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EP 0 395 853 A1(54) **Recombinant Interleukin-2 receptor.**

(57) **Recombinant IL-21R β chain or portions thereof, cDNA coding therefore, vectors containing said cDNA, hosts transfected by said vectors, and monoclonal antibodies to said recombinant IL-2R β or portions thereof.**

Recombinant Protein Receptor

This invention relates to receptors for interleukin-2, more particularly to the β -chain of the receptor, and to cDNA coding for the β -chain or parts thereof, vectors containing cDNA inserts coding for the β -chain, hosts transformed by such vectors and the cultivation of such hosts to produce the said β -chain.

Ample evidence has been accumulated that cytokines, a class of soluble mediators involved in cell-to-cell "communications", are essential in the regulation of the immune system. It has been known that cytokines induce proliferation, differentiation and activation of target cells through interaction with specific cell surface receptor(s). Interleukin-2 (IL-2), previously defined as T cell growth factor (1), is one of the best characterized cytokines, known to play a pivotal role in the antigen-specific clonal proliferation of T lymphocytes (T cells) (2). IL-2 also appears to act on other cells of the immune system such as immature thymocytes (3), B lymphocytes (B cells) (4), macrophages (5), natural killer cells (NK cells) (6), and lymphokine-activated killer cells (LAK cells) (7). These multifunctional properties of IL-2 have now opened up possibilities in the formulation of immunotherapies such as adoptive immunotherapy (8). More recently, IL-2 has been shown to function also on neural cells such as oligodendrocytes (9), suggesting a possible involvement of this cytokine in the central nervous system. Despite extensive studies on the IL-2 system in the context of basic and clinical immunology, information has been limited on the molecular mechanism(s) underlying the IL-2-mediated signal transduction (10).

The IL-2 receptor (IL 2R) is known to be unique in that it is present in three forms: high-, intermediate- and low-affinity forms with respect to its binding ability to IL-2, and respective dissociation constants (Kds) of 10^{-11} M, 10^{-9} M and 10^{-8} M (11, 12). Following the characterization of IL-2R α chain (Tac antigen, p55) (13), it became evident that the α chain constitutes solely the low-affinity form and it is not functional per se in IL-2 internalization and signal transduction, unless associated with another specific membrane component(s) of lymphoid cells (14, 15). Subsequently, the lymphoid membrane component was identified to be a novel receptor chain, termed β chain (or p70-75) (12, 16, 17). In fact, experimental evidence has suggested that the IL-2R β chain per se constitutes the intermediate-affinity form (12). In addition, its association with the IL-2R α chain results in the high-affinity form of the receptor (12, 16, 17). Expression studies using wild type and mutated IL-2R α chain cDNAs strongly support the notion that the IL-2R β chain but not the IL-2R α chain possesses a domain(s) responsible for driving the intracellular signal transduction pathway(s) (18). There exists, therefore, a need to obtain IL-2 β chain in amounts which will enable its structure and function to be elucidated, this being an essential step in gaining further insight into the molecular basis of the high-affinity IL-2R as well as on the mechanism of signal transduction operating in IL-2 responsive cells. To this end we describe below cDNA coding for the IL-2R β chain or parts thereof whereby insertion of said cDNA in a suitable vector and expression thereof in an appropriate host will enable recombinant and large scale production of protein corresponding to the IL-2R β chain or parts thereof.

According to a one aspect of the present invention therefore we provide a recombinant cDNA coding for an IL-2R β chain or a portion thereof.

cDNA of the invention may have the formula:

ATG

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GCG GCC CCT GCT CTG TCC TGG CGT CTG CCC CTC CTC ATC
 CTC CTC CTG CCC CTG GCT ACC TCT TGG GCA TCT GCA GCG
 GTG AAT GGC ACT TCC CAG TTC AGA TGC TTC TAC AAC TCG
 AGA GCC AAC ATC TCC TGT CTC TGG AGC CAA GAT GGG GCT
 CTG CAG GAC ACT TCC TGC CAA GTC CAT GCC TGG CCG GAC
 AGA CGG CGG TGG AAC CAA ACC TGT GAG CTG CTC CCC GTG
 AGT CAA GCA TCC TGG GCC TGC AAC CTG ATC CTC GGA GCC
 CCA GAT TCT CAG AAA CTG ACC ACA GTT GAC ATC GTC ACC
 CTG AGG GTG CTG TGC CGT GAG GGG GTG CGA TGG AGG GTG
 ATG GCC ATC CAG GAC TTC AAG CCC TTT GAG AAC CTT CGC
 CTG ATG GCC CCC ATC TCC CTC CAA GTT GTC CAC GTG GAG
 ACC CAC AGA TGG AAC ATA AGC TGG GAA ATC TCC CAA GCC
 TCC CAC TAC TTT GAA AGA CAC CTG GAG TTC GAG GCC CGG
 ACG CTG TCC CCA GGC CAC ACC TGG GAG GAG GCC CCC CTG
 CTG ACT CTC AAG CAG AAG CAG GAA TGG ATC TGC CTG GAG
 ACG CTC ACC CCA GAC ACC CAG TAT GAG TTT CAG GTG CGG
 GTC AAG CCT CTG CAA GGC GAG TTC ACG ACC TGG AGC CCC
 TGG AGC CAG CCC CTG GCC TTC AGG ACA AAG CCT GCA GCC
 CTT GGG AAG GAC ACC ATT CCG TGG CTC GGC CAC CTC CTC
 GTG GGC CTC AGC GGG GCT TTT GGC TTC ATC ATC TTA GTG
 TAC TTG CTG ATC AAC TGC AGG AAC ACC GGG CCA TGG CTG
 AAG AAG CTC CTG AAG TGT AAC ACC CCA GAC CCC TCG AAG
 TTC TTT TCC CAG CTG AGC TCA GAG CAT GGA GGA GAC GTC
 CAG AAG TGG CTC TCT TCG CCC TTC CCC TGA TCG TCC TTC
 AGC CCT GGC GGC CTG GCA CCT GAG ATC TCG CCA CTA GAA
 GTG CTG GAG AGG GAC AAG GTG ACG CAG CTG CTC CTG CAG

5 CAG GAC AAG GTG CCT GAG CCC GCA TCC TTA AGC AGC AAC
CAC TCG CTG ACC AGC TGC TTC ACC AAC CAG GGT TAC TTC
TTC TTC CAC CTC CCG GAT GCC TTG GAG ATA GAG GCC TGC
CAG GTG TAC TTT ACT TAC GAC CCC TAC TCA GAG GAA GAC
CCT GAT GAG GGT GTG GCC GGG GCA CCC ACA GGG TCT TCC
CCC CAA CCC CTG CAG CCT CTG TCA GGG GAG GAC GAC GCC
10 TAC TGC ACC TTC CCC TCC AGG GAT GAC CTG CTG CTC TTC
TCC CCC AGT CTC CTC GGT GGC CCC AGC CCC CCA AGC ACT
GCC CCT GGG GGC AGT GGG GCC GGT GAA GAG AGG ATG CCC
CCT TCT TTG CAA GAA AGA GTC CCC AGA GAC TGG GAC CCC
15 CAG CCC CTG GGG CCT CCC ACC CCA GGA GTC CCA GAC CTG
GTG GAT TTT CAG CCA CCC CCT GAG CTG GTG CTG CGA GAG
GCT GGG GAG GAG GTC CCT GAC GCT GGC CCC AGG GAG GGA
20 GTC AGT TTC CCC TGG TCC AGG CCT CCT GGG CAG GGG GAG
TTC AGG GCC CTT AAT GCT CGC CTG CCC CTG AAC ACT GAT
GCC TAC TTG TCC CTC CAA GAA CTC CAG GGT CAG GAC CCA
25 ATC CAC TTG GTG TAG

which codes for human II-2R β , or a degenerate variant thereof or a portion thereof.

Another cDNA of the invention for instance has the formula:

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GCT ACC ATA GCT CTT CCC TGG AGC CTG TCC CTC TAC GTC TTC CTC CAG GCC AAT GTC TCT TGC ATG TGC ACC CAT GAA
 GCA GTC AAA AAC TGT TCC CAT CTT GAA TGC TTC TAC AAC TCA AGA GCC AAT GTC TCT TGC ATG TGC ACC CAT GAA
 GAG GCT CTC AAT GTC ACA ACC TGC CAC GTC CAT GCC AAG TCG AAC CTC CGA CAC TCG AAC AAA ACC TCT GAG CTA
 ACT CTT GTG AGG CAG GCA TCC TGG GCC TGC AAC CTC ATC CTC GGG TCG TTC CCA GAG TCC CAG TCA CTG ACC TCC
 CTG GAC CTC CTT GAC ATA AAT GTG GTG TGC TGC TGG GAA GAG AAG GGT TGG CCT AGG CTA AAG ACC TGC GAC TTC CAT
 CCC TTT GAC AAC CTT CGC CTG GTG GCC CTT CAT TCC CTC CAA CTT CTC CAC AAT GAT ACC CAG AGA TGT AAC ATA
 AGC TGG AAG GTC TCC CAG GTC TCT CAC TAC AAT CAA CCA TAC TTG GAA TTT GAG GCC CGT AGA CGT CTT CTG GGC
 CAC AGC TGG GAG CAT GCA TCC GTA TTA AGC CTC AAG CAG AGA CAG AGC TGG CTC TTC TTG GAG ATG CTG ATC CCT
 AGT ACC TCA TAT CAG CTC CAG GTG AGG GTC AAA GCT CAA CGA AAC AAT ACC GGG ACC TGG AGT CCC TGG AGC CAG
 CCC CTC ACC TTT CGG ACA AGG CCA GAA GAT CCC ATG AAG CAG ATC CTC CCC ATC TCA TGG CTC AGA TAC CTT CTC
 CTG GTC CTT GCT TGT TTT TCT GGC TTC TCC TGC GTC TAC AAT TTG CTC AAG XXX CGG TAC CTT GCG CCA TGG

XXX = CGC or TCC

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The present invention thus also includes within its scope cDNA coding for portions of the above

mentioned cDNAs, e.g. portions of the complete sequence of the hIL-2R β chain, for instance the extracellular portion beginning at, or about amino acid (a.a) (see Fig. 1 B) 1 e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 and ending at or about a.a. 214 e.g. 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 214, 215, 216, 217, 218, 219, 220, or sub-portions of this extracellular part or degenerate variants thereof, or portions corresponding to the intracellular part of the receptor chain e.g. the portion beginning at or about a.a. 239 e.g. a.a. 230, 231, 232, 234, 235, 236, 237, 238, 239, 240, 241, 242, up to or about the end a.a. 525, e.g. 516, 517, 518, 519, 520, 521, 522, 523, 524 and 525 or sub-portions thereof, or degenerate variants thereof, as well as cDNA coding for portions of the complete sequence of the murine IL-2R β chain (see Fig. 8) for instance the extracellular part beginning at, or about amino acid 1 e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 and ending at or about amino acid 210 e.g. 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220 or sub-portions of this extracellular part or degenerate variants thereof, or portions corresponding to the intracellular part of the receptor chain e.g. the portion beginning at or about amino acid 235 e.g. amino acid 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, up to or about the end a.a. 513 e.g. 505, 506, 507, 508, 509, 510, 512, and 513 or sub-portions thereof or degenerate variants thereof.

It will be understood that for the particular IL-2R β chains or portions thereof described herein, natural allelic variations may exist, occurring from individual to individual. These variations may be demonstrated by one or more amino acid differences in the overall sequence or by deletions, substitutions, insertions, inversions or additions of one or more amino acids in the sequence. In addition it will be understood that the IL-2R β chain or portions thereof described herein may be modified by genetic engineering techniques e.g. point mutation, for the substitution, deletion or addition of one or more amino acids without changing the essential characteristics of the IL-2R β chain or portion thereof. The present invention thus also includes within its scope DNA sequences capable of hybridising with the DNA sequences described herein and coding for proteins having substantially the activity of an IL-2R β chain or portions thereof, especially soluble IL-2R β .

In one further aspect of the invention we provide a recombinant DNA molecule coding for a water soluble portion of the human IL-2R β (hIL-2R β) for example an amino acid sequence comprising the amino acids about 1 to about 210 of the entire hIL-2R β . Such a DNA molecule may code, for example, for a soluble human interleukin 2 receptor β -chain derivative having 212 amino acid residues in which residues 1 to 210 correspond to the amino acids of the native IL-2R β -chain.

For example in one embodiment described below the terminal nucleotides of a cDNA molecule coding for a soluble hIL-2R β derivative are as follows

GCC CTT GCT AGC TAG
208 Ala Leu Ala Ser [Stop]

Using standard techniques of recombinant DNA technology vectors for transforming suitable host cells can be constructed which contain cDNA sequences corresponding to the structural gene for IL-2R β as set forth above or any desired portion thereof, or degenerate variant thereof.

Suitable vectors are plasmid vectors for example and will include control and regulatory sequences operably linked to the cDNA sequence coding for the IL-2R β chain or portion thereof.

Suitable techniques are well known and widely practised and by way of Example are described, in connection with other proteins, in European Patent Applications, Publication Nos. 0254249 and 0170204.

Obtaining the desired protein in pure form from the culture can be carried out by standard techniques and such protein provides a suitable antigen for preparing monoclonal antibodies. Thus hybridomas capable of secreting a monoclonal antibody having a specific affinity to the IL-2R β chain or a desired portion thereof may be prepared by immunizing a non-human animal with recombinant IL-2R β or a portion thereof, removing spleen cells with non-immunoglobulin secreting myeloma cells, and selecting from the resulting hybridomas a cell line which produces a monoclonal antibody having the desired binding specificity and, if desired, subsequently sub-cloning said hybridoma.

The techniques for preparing hybridomas and obtaining monoclonal antibodies in pure form therefrom are well known and by way of example are described in European Patent Application, Publication No. 0168745.

Antibodies in accordance with the invention are useful e.g. for diagnostic purposes and also for therapy by immune suppression or activation. As mentioned above, such antibodies could be raised using purified recombinant protein in accordance with the invention or by transfecting the cDNA of the invention, obtaining

cells expressing large amounts of the receptor and using such cells to obtain the antibodies.

As indicated above the present invention envisages soluble forms of IL-2R β chain and of soluble IL-2 receptor. Soluble forms include those coded for by the partial cDNA sequences coding for the extracellular part of IL-2R β or subportions thereof as described above. If desired both IL-2R β chain and α -chain may be produced simultaneously.

The availability of monoclonal antibodies to specific sub-portions of the IL-2 β chain enables epitopes of the receptor chain to be identified and thus opens the way for control of the activity of the receptor to be exercised using suitable monoclonal antibodies or other peptides or peptidomimetic or protein analogue substances.

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Isolation and analysis of the cDNA clones coding for human IL-2R β chain

In isolating the cDNA clones, an expression cloning strategy was applied by using the monoclonal antibodies, Mik- β 1 and Mik- β 2 (19), both of which have been raised against the IL-2R β chain found on the human leukemic cell line YT (20).

The monoclonal antibodies Mik- β 1 and Mik- β 2 are both deposited at Fermentation Research Institute, Agency of Industrial Science and Technology, Japan. The deposit numbers for Mik- β 1 and Mik- β 2 are, 10453 and 10454 (1988), respectively; they are also described in Japanese Patent Application No. 298742 (1988).

A few sets of cDNA libraries were prepared by using the poly(A)⁺-RNA from YT cells according to standard procedures. cDNA libraries were prepared with CDM8 vector according to published procedures (21), except using random primer (Amersham) or oligo (dT) primer as mentioned below. The plasmid DNA representing 5.6×10^6 independent colonies was prepared by the standard procedure and one mg of DNA was used for the first DNA transfection. Actually, the DNA was divided into 100 tubes (therefore each tube contained 10 μ g of DNA) and they were each transfected into 3.5×10^5 monkey COS cells in a tissue culture dish (60 mm polystyrene dish, Corning). The transfection was done using the standard DEAE dextran procedures. The transfected COS cells were then treated with the cocktail of Mik- β 1 and - β 2 antibodies (400-fold diluted ascites for each antibody) and subjected to the standard panning procedure. The dish used for the panning was FALCON 60 mm dish, coated with anti-mouse IgG as described previously (ref. 21). In this first round of panning, 100 IgG-coated dishes were used. After the panning, Hirt extract was prepared by the standard procedure (ref. 21) and the recovered plasmids were introduced into E.coli by the method described in ref. 21. By this procedure 3.7×10^6 colonies were obtained. Those bacterial colonies were fused with COS cells by the standard protoplast fusion procedures (ref. 21). In these fusion experiments, 26 Corning dishes each containing 5×10^5 COS cells were used. After the fusion, the COS cells were subjected to panning as described above and Hirt extract was prepared. 32,000 bacterial colonies were obtained from the Hirt extract. The fusion, panning procedures were repeated again and 32,000 bacterial colonies were obtained from the subsequent Hirt extract. The same procedures were repeated once again, obtaining 28,000 bacterial colonies (in the meantime, there should be a dramatic enrichment of the objective clones). The same procedures were repeated once again and 6,000 colonies were obtained. From these colonies, 30 colonies were picked up randomly and the cDNA inserts were analysed. Of them, only 7 colonies contained plasmids from which cDNA inserts can be excised by restriction enzyme XhoI. The vector derived XhoI sites were located at both sides of the cDNA and all other plasmids had lost such cleavage sites due to the DNA rearrangements; in fact, all of them were much smaller in size than the original vector. Thus they were considered to be non-specific products. On the other hand, all of the 7 colonies were derived from the same mRNA, as confirmed by the conventional restriction enzyme cleavage analysis and DNA blot analysis. Of them, one plasmid, termed pIL-2R β 30 contained longer cDNA than the other 6 plasmids which turned out to be identical to each other (designated pIL-2R β 9).

In this procedure, therefore, we isolated two independent cDNA clones, pIL-2R β 9 and pIL-2 β 30; each of the expression products specifically reacted with the antibodies. The two clones contained cDNA inserts of 1.3Kb and 2.3Kb, respectively, and cross-hybridized with each other. Subsequent sequence analysis of the cDNAs revealed that they represent the same mRNA. In fact, RNA blotting analysis revealed that the mRNA is approximately 4Kb in size (see below). Subsequently, we screened other YT cDNA libraries by using the cloned cDNAs as probes, and several independent cDNA clones which together cover the entire mRNA for the IL-2R β chain were isolated. Thus pIL-2R β 6 and pIL-2R β 19 were obtained by screening the cDNA libraries with the pIL-2R β 9 cDNA insert in the probe.

The above mentioned plasmids containing cDNA coding for human IL-2R β sequences have been deposited in strain E.coli MC 1061/P3 on March 2, 1989 at the Fermentation Research Institute according to

the Budapest Treaty under the following accession numbers:

Plasmid	Accession No.
pIL-2R β 6	FERM BP-2312
pIL-2R β 9	FERM BP-2313
pIL-2R β 19	FERM BP-2314
pIL-2R β 30	FERM BP-2315

The complete nucleotide sequences of four of the cloned cDNAs were determined (Fig. 1).

Fig. 1 shows the structure of the human IL-2R β chain cDNA. Fig. 1a is a schematic representation of the mRNA as deduced from the cloned cDNAs. Dotted, hatched, open and closed rectangles representing respectively the signal sequence, the extracellular, the transmembrane and the cytoplasmic regions of the mRNA are shown below. Fig. 1b shows the nucleotide and amino acid sequences of the human IL-2R β chain cDNA. The sequence was deduced following the complete DNA sequence analysis of the above described cDNA clones. Nucleotides are numbered on the right margin and amino acids are numbered on the left margin. Clones pIL-2R β 19 and pIL-2R β 6 each contained G-A mutation at nucleotide residues 425 and 1531, respectively. Thus pIL-2R β 6 cDNA acquired a TAG triplet in the cytoplasmic region. It is thought to be an error in reverse transcription, since all other clones, pIL-2R β 30, pIL-2R β 19 and pIL-2R β 16, have a TGG triplet at that position. The first underlined 26 amino acid residues represent the signal sequence as predicted by the consensus sequence (22). The 25 transmembrane amino acid residues are marked with a thick underlining. The cysteine residues are boxed. The potential N-glycosylation sites are underlined twice. The possible poly-adenylation signals are shown by open rectangle. In summary, RNA was prepared from the NK-like human lymphoid cell line, YT, and cDNA libraries were prepared with CDM8 vector according to published procedures (21), except using either random primers (Amersham) (for pIL-2R β 6, 9 and 30), or oligo (dT) primer (for pIL-2R β 19). Screening of the cDNA libraries by a cocktail of anti-IL-2R β monoclonal antibodies, Mik- β 1 and Mik- β 2, was carried out as described previously (21). Nucleotide sequences were determined by a combination of dideoxy chain termination and chemical cleavage methods.

As shown in Fig. 1, the cDNA contains a large open reading frame that encodes a protein consisting of 551 amino acids. No significant homology with other known proteins was found in the protein Sequence Database (National Biomedical Research Foundation, Washington, D.C.) or with sequences published more recently. Unlike many of other cytokine receptors, it appears that IL-2R α and IL-2R β chains do not belong to the immunoglobulin superfamily. From the deduced structure of the protein, the N-terminal 26 amino acids is considered to represent the signal sequence. Thus the mature form of the IL-2R β chain consists of 525 amino acids with a calculated M.W. of 58.358. As shown in Fig. 1, the receptor molecule consists of an extracellular region consisting of 214 amino acids. This region contains 8 cysteine residues of which 5 residues are found in the N-terminal half and they are interspaced rather periodically by 9-12 amino acids. It is likely that disulfide linkages between the cysteine residues impart a stable configuration for ligand binding. In fact, abundance of cysteine residues seems to be one of the common features of the ligand binding domain of many receptors (23). It may be worth noting that the predicted number of amino acids (a.a.) within the extracellular region of the IL-2R β chain (214 a.a.) is almost comparable in number to that of the IL-2R α chain (219 a.a.). Such size similarity may be significant in considering the conformation of the heterodimeric receptor complex that is quite unique for this receptor; as both α and β chains individually interact with distinct sites of the same IL-2 molecule (24).

A hydrophobic stretch of 25 amino acids spanning from the 215 to 239 amino acid residues appears to constitute the membrane spanning region of the receptor (Fig. 1 and 2).

Fig. 2 is a hydropathy plot analysis of deduced human IL-2R α and IL-2R β chain precursor structures. The analysis was carried out according to Kyte and Doolittle (38). SG and TM represent signal sequence and transmembrane sequence respectively.

The cytoplasmic region of the β chain consists of 286 a.a. and it is far larger than that of the α chain, which is only 13 a.a. long. The consensus sequences of tyrosine kinase (Gly-x-Gly-x-x-Gly) (25) are absent in the β chain. However, the presence of a triplet, Ala-Pro-Glu (293-295) may be noted; this has been asserted to be the consensus motif for a catalytic domain of some protein kinases (25). The possibility of the cytoplasmic region of the β chain having a protein kinase activity has yet to be tested. The primary structure of this region revealed yet another interesting feature; a rather strong bias for certain characteristic amino acids. This region is rich in proline (42/286) and serine (30/286) residues. Interestingly, the "proline rich" structure has also been demonstrated in the cytoplasmic region of CD2, a T cell membrane antigen

involved in the activation pathway of T cells (26). The proline-rich structure may impart a non-globular conformation to this region that may be important in coupling of the receptor molecule with other signal transducer(s). The predominant serine residues may be the major target for phosphorylation, which could also modulate the receptor function (27). In addition, the cytoplasmic region is notably biased for negatively charged amino acids. In fact, this region contains 40 such amino acids (i.e. glutamic and aspartic acids), whereas only 18 amino acids account for the positively charged residues (i.e. lysine and arginine). Such a bias is particularly notable in the middle portion (a.a. 345-390) of the cytoplasmic region. Thus, the cytoplasmic region of the β chain may be quite acidic. Taken together some, if not all, of these unique characteristics may be responsible in driving further the downstream signal transduction pathway(s). The receptor protein contains 5 potential sites for N-linked glycosylation (Fig. 1B), of which 4 are located in the extracellular region. Such a posttranslational modification may account for the difference between the M.W. of the estimated mature (70-75kD) and the calculated (58kD) protein molecules. Hydropathy plot analysis of the α and β chains revealed the presence of hydrophilic regions just adjacent to the cell membrane in the both chains (Fig. 2) These regions may play a role in the non-covalent intramolecular association between the two chains.

Expression of Human IL-2R β chain mRNA

Expression of the IL-2R β mRNA was examined by using the cDNA insert from pIL-2R β 30 as the probe. Fig. 3a illustrates the expression of human IL-2R β chain mRNA in different cell types. Poly(A)⁺ RNA (2 μ g per lane) from the following cell sources was prepared and subjected to RNA blotting analysis using the XhoI-digested human IL-2R β chain cDNA fragment derived from pIL-2R β 30 as a probe following standard procedures (14, 18, 27). Lane 1, YT; lane 2, Hut102(HTLV-1 transformed human T cell line); lane 3, MT-2(HTLV-1 transformed human T cell line); lane 4, ARH-77 (multiple myeloma line); lane 5, SKW6.4 (EBV-transformed human B lymphoblastoid line); lane 6, U937 (histiocytic leukemia line); lane 7, MT-1 (HTLV-1 transformed human T cell line); lane 8, Jurkat (human T leukemic line); lane 9, HeLa (human cervical carcinoma cell line).

As shown in Figure 3a, the RNA blot analysis revealed the presence of a 4kb mRNA, the expression of which is restricted to lymphoid cells previously identified to bear IL-2R β chain (i.e. YT, MT-2, Hut102, SKW6.4) (12, 16, 17). On the other hand, the mRNA expression was not detected in cells such as Jurkat, MT-1, U937, ARH-77 and HeLa cells. Essentially, the mRNA expression levels are in correlation with the IL-2R β chain expression levels.

Fig. 3 b illustrates the expression of IL-2R β and IL-2R α mRNAs in human PBLs. Total RNA (15 μ g per lane) was loaded in each lane. Lanes 1 and 4 represent unstimulated human peripheral blood lymphocytes (PBLs); lanes 2 and 5, PBLs stimulated with 5 μ g/ml phytohemagglutinin (PHA) for 24 hrs; lanes 3 and 6, PBLs stimulated with 5 μ g/ml PHA for 72 hrs. The RNA-blotted filter was hybridized with the IL-2R β probe (lanes 1-3). After dehybridization of the IL-2R β probe, the same filter was hybridized with the IL-2R α probe (XbaI-BclI fragment derived from pSVIL2R-3 (14) (lanes 4-6).

Interestingly, the IL-2R β mRNA was detectable in the unstimulated PBLs and its expression levels increased transiently only 2.5-fold after mitogen stimulation. Based on previous data derived from flow cytometric analysis (19), it is likely that the mRNA induction patterns differ between the different lymphocyte populations. This expression pattern is quite different from that of the IL-2R α chain whose expression strictly requires mitogenic stimulation of the cells (Fig. 3b), suggesting the presence of distinct mechanisms of gene expression between the two genes.

Southern blot analysis of the genomic DNA from PBL and various cell lines including HTLV-1-transformed human T cell lines indicates that the gene is present in a single copy and is not rearranged in those cells.

IL-2 binding properties of the cDNA-encoded IL-2R β chain

We next carried out a series of cDNA expression studies in order to examine whether the cDNA product binds IL-2 and indeed manifests the properties of the IL-2R β chain that have been demonstrated and/or suggested in previous studies. Two cDNA expression plasmids were constructed in which expression of the cDNA spanning the entire coding region was directed by either the mouse *lck* gene (29) promoter (pLCKR β) or Moloney leukemia virus LTR (30) (pMLVR β).

Expression vectors were constructed by the following procedures. pIL-2R β 30 was digested with HindIII

(the cleavage site is located within the polylinker regions of CDM8) and, after fill-in of both ends, a BamHI linker was attached and religated. The resulting plasmid was then digested with BamHI and the 1.8kb DNA fragment which contains the entire coding sequence for the β chain was introduced into BamHI-cleaved p1013 vector containing the mouse lck promoter to construct pLCKR β . The BamHI-digested cDNA fragment was also introduced into a retrovirus vector, pZipSV(X) (30), to construct pMLVR β . The human IL-2R α expression vector, pSVIL2Rneo, was obtained from pSVIL2R-3 (14) by replacing the Eco-gypt gene with the neo-resistance gene.

The plasmid pLCKR β was introduced into the mouse T lymphoma EL-4 and the human T cell leukemia Jurkat lines, both of which are known to be devoid of surface molecules that bind human IL-2.

Transfection of the expression plasmids into Jurkat and EL-4 cells was carried out by electroporation as described previously (39). Transfected cells were selected in the RPMI1640 medium containing 10% fetal calf serum (FCS) and G418 (1 mg/ml for EL-4 and 1.5 mg/ml for Jurkat). To obtain cells expressing cDNAs for human IL-2R α and IL-2R β chains simultaneously, a Jurkat-derived clone J α -5, transfected with pSVIL2Rneo, was co-transfected with pLCKR β and a plasmid containing the hygromycin-resistance gene, pHgy. The transfected cells were selected with 200 μ g/ml hygromycin. Transfection of pMLVR β into 2 cells was carried out by calcium-phosphate method as described previously (14) and the cells were selected by 700 μ g/ml of G418. For flow cytometric analysis, 5×10^5 cells were treated with antibody (1:500 dilution of ascites) at 4°C for 30 min. After washing, cells were stained with fluorescein-conjugated goat anti-mouse IgG.

The stained cells were analysed on a FACS440 flow cytometer (Beckton Dickinson). The 125 I-IL-2 binding assay and Scatchard plot analysis were carried out as described previously (12).

Fig. 4a illustrates the expression of human IL-2R α and/or IL-2R β chain cDNAs by means of cell surface staining patterns of human IL-2R α and/or IL-2R β cDNA transfectants. Parental cells and various transfectant cells were separately stained with either a monoclonal anti-human IL-2R α antibody, anti-Tac (-----), or monoclonal anti-human IL-2R β antibody, Mik- β 1 (———). Dotted line (.....) is a fluorescence profile of the cells stained with fluorescein-conjugated goat-anti-mouse IgG alone. Cells used were (1) EL β -13 (and EL-4-derived clone transfected with pLCKR β), (2) J β -8 (a Jurkat-derived clone transfected with pLCKR β), (3) J α -5 (a Jurkat-derived clone transfected with pSVIL2Rneo), (4) J α -2 (a J α -5-derived clone transfected with pLCKR β), (5) J α β -10 (a J α -5-derived clone transfected with pLCKR β), and (6) F β -3 (a NIH3T3-derived line transfected with pMLVR β).

Stable transformant clones expressing the cDNA product were obtained for both the EL-4 (EL β -13) and Jurkat (J β -8) cells as judged by FACS analysis (Fig. 4a). In addition, we also introduced the same gene into the Jurkat transformant clone, J α -5, which expresses the transfected, human IL-2R α chain cDNA. Two of the resulting transformants, J α β -2 and J α β -10, were found to express both α and β chains (Fig. 4a-(4), (5)). As expected, RNA blotting analyses of the mRNA expressed in those transformants revealed that the α and β chain-specific mRNAs are derived from the transfected cDNAs but not from the endogeneous genes (26). Furthermore, in order to examine the property of the cDNA product in non-lymphoid cells, the plasmid pMLVR β was introduced into an NIH3T3 cell-derived cell line 2 (30), and the resulting transformant expressing the cDNA, F β -3, was obtained (Fig. 4a-(5)).

The IL-2 binding studies were carried out with 125 I-labeled, recombinant human IL-2.

Fig. 4b illustrates the expression of the α and β chains by means of the Scatchard plot analysis of 125 I-IL-2 binding to the transfectants expressing the cloned cDNAs. Scatchard plot of the IL-2 binding data in the absence (-o-o-) or presence (-●-●-) of 1:100-diluted ascites of Mik- β 1. Binding of 125 I-IL-2 to EL β -13 or J β -8 was completely abolished by Mik- β 1. No specific IL-2 binding was observed when parental Jurkat or EL-4 cells were examined. The number of IL-2 binding sites per cell and the receptor affinity were determined by computer-assisted analysis of the IL-2 binding data. (1) EL β -13, (2) J β -8, (3) J α -5, (4) J α β -2, (5) J α β -10.

As can be seen the EL-4-derived clone (EL β -13) and the Jurkat-derived clone (J β -8), both expressing the β chain cDNA displayed intermediate-affinity to IL-2 with estimated Kd values of 4.0nM and 2.7nM, respectively. The IL-2 binding to those cells was completely abolished by the Mik- β 1 antibody (Fig. 4b-(1), (2)). The Jurkat-derived J α β -2 and J α β -10 clones expressing both the human IL-2R α and IL-2R β cDNA displayed both high and low affinity receptors with estimated Kp values of 22pM and 15nM for J α β -2 and 19pM and 33nM for J α β -10, respectively. In contrast, the parental, Jurkat-derived J α -5 cells expressing the α chain cDNA alone manifested exclusively low-affinity (Kd: 19.5nM) to IL-2 (Fig. 4b-(3)). The number of the high-affinity IL-2R expressed J α β -2 cells and J α β -10 was comparable to that of expressed IL-2R β molecules. In addition, treatment of these cells with Mik- β 1 antibody completely abolished high-affinity IL-2 binding sites from the cell surface, while retaining the expression of low-affinity IL-2R (Fig. 4b-(4), (5)). These observations demonstrate unequivocally that the cDNA-encoded IL-2R β molecule is directly involved in the formation of high-affinity receptor complex in association with the IL-2R α chain. In contrast to the

aforedescribed T cell transformants, the F β -3 cells did not display any IL-2 binding on the cell surface under same binding conditions. Interestingly the same observation was made with monkey COS cells that express the β chain, but failed to bind IL-2 (28). Thus, the results suggest the involvement of either a cell-type specific processing mechanism(s) or an additional cellular component(s), or both for the functional IL-2R β chain expression.

In order to characterize further the molecular structure of reconstituted IL-2R, we performed chemical crosslinking experiments with 125 I-IL-2 and non-cleavable chemical crosslinker, dissuccinimidyl suberate (DSS).

Fig. 5 illustrates the results of the affinity crosslinking studies of the IL-2R-positive transformants. Cells were incubated with 5nM (lanes 1-13) or 100pM (lanes 14-16) of 125 I-IL-2 in the absence (lanes 1-4, 14-16) or presence of a 250-fold molar excess of unlabeled IL-2 (lanes 5-7), 500-fold molar excess of affinity column-purified Mik- β 1 (lanes 8-10) or 500-fold molar excess of affinity column-purified anti-Tac (lanes 11-13). Then cells were chemically crosslinked with dissuccinimidyl suberate (DSS) as described previously (16). The cells were then solubilized and the supernatants were subjected to 7.5% SDS-PAGE. Cells used were: Jurkat (lane 1); J α -5 (lanes 2, 5, 8, 11, 14); J β -8 (lanes 3, 6, 9, 12, 15); J $\alpha\beta$ -10 (lanes 4, 7, 10, 13, 16). YT cells crosslinked with 125 I-IL-2 were used as a marker (M).

As can be seen cells expressing only IL-2R β chain were crosslinked with 125 I-labeled IL-2 and analysed by SDS-PAGE, a doublet band consisting of 90kD major and 85kD minor was detected and its migration profile was indistinguishable from that of YT cells (see arrows in Fig. 5 and ref. 16, 17). The appearance of the doublet is inhibited by an excess of unlabeled IL-2 or by Mik- β 1. The doublet formation may be due to degradation of receptor-IL-2 complex. It is also possible that both protein products are derived by a differential post-translational modification(s). Alternatively, one of the doublet may represent a third component of the receptor complex. A broad band migrating around the position of 150kD was also detected in the transfectant (J $\alpha\beta$ -10) as well as YT cells. The appearance of this band is also inhibited by either unlabeled IL-2 or Mik- β 1. It may represent the ternary complex of IL-2, IL-2R α and IL-2R β molecules. In a series of chemical cross-linking experiments shown in Fig. 4, it was demonstrated that the physico-chemical properties of the receptor complex expressed on the surface of J $\alpha\beta$ -2 are indistinguishable from the properties of high-affinity receptor expressed on cultured T cells or PBLs (12, 16, 17).

Preliminary results of experiments to determine whether the expression of the α and β chains in non-lymphoid cells results in the formation of high-affinity receptor indicate that, when the α and β chain cDNAs are co-expressed transiently in COS cells, both chains can crosslink with 125 I-IL-2 at the concentration (400 pM) in which the similarly expressed α chain alone can not (28). The results may suggest the formation of the $\alpha\beta$ heterodimeric receptor in this non-lymphoid cell line.

IL-2 internalization by reconstituted receptors

It has been reported that intermediate- and high-affinity IL-2 receptors can both internalize IL-2 (33-35). Ligand internalization is usually accompanied with the IL-2 signal transduction, suggesting this process to be essential.

Fig. 6 illustrates IL-2 internalization via the reconstituted receptors. IL-2 internalization was examined according to a method described previously (33). Briefly, cells (5×10^7) were treated with 125 I-IL-2 at a final concentration of 200pM (J $\alpha\beta$ -10) or 5nM (J α -5, J β -8 and EL β -13) at 0°C for 30 min. After washing, cells were suspended with prewarmed culture medium (37°C) and the kinetics of IL-2 internalization was examined as described previously (33). (a) EL β -13, (b) J β -8, (c) J $\alpha\beta$ -10, (d) J α -5. (—●—●—), internalized IL-2; (...o...o...), cell-surface bound IL-2; (—■—■—■—), free IL-2.

As shown in Fig. 6, we examined whether the reconstituted receptors can internalize IL-2. In fact, the cells expressing IL-2R β chain alone, or both α and β chains are capable of internalizing IL-2 following a kinetic pattern similar to that reported for the native receptor. In contrast, the Jurkat cells expressing only IL-2R α failed to internalize IL-2, similar to previously reported observations (33, 34). Preliminary results indicate that the growth of the cells expressing the intermediate- or high-affinity receptors is selectively inhibited by IL-2 (14, 36). We also have preliminary results that the β chain expressed in another host cell line functions in stimulating the cell growth in response to IL-2 (28).

Cloning of Murine IL-2R receptor β chain

No specific antibodies to murine IL-2 receptor β -chain are known to exist, accordingly the screening

method used for the isolation of cDNA for Hu IL-2R β chain was not employed.

A cDNA library was prepared using poly (A)⁺-RNA from Concanavalin A stimulated mouse spleen cells; the cDNA was cloned in λ gt 10 which was multiplied in *E. coli*.

Screening of this library was then carried out using the above described human IL-2R β chain cDNA as the probe under non-stringent conditions. From the positive clones a clone designated λ MIL 2R β -26 was selected. The cDNA insert in this clone contained only a 540 bp sequence of the whole murine IL-2R β chain sequence. This sequence was therefore isolated by digestion of λ MIL-2R β -26 using Pvu 2 and used for screening another cDNA library prepared using poly (A)⁺ from the mouse thymoma cell line EL-4 according to standard procedures and cloned into the BstXI site of the CDM8 vector followed by transfecting *E. coli*.

Screening of the cDNA library was carried out under highly stringent conditions according to the method described in European Patent Application No. 88 119 602.9 and Kashima et al. (Nature Vol. 313 pp 402-404, 1985).

From the positive clones clone pMIL-2R β -36 containing the structural gene for murine IL-2R β (see Fig. 8) was selected. The restriction map of the cDNA clone is shown in Fig. 7.

Plasmid pMIL-2R β -36 has been deposited in strain *E. coli* MC 1061/P3 on May 23, 1989 at the Fermentation Research Institute according to the Budapest Treaty under accession number FERM BP-2435.

Preparation of soluble human interleukin 2 receptor β -chain.

A secreted form of the hIL2-R β chain (termed hereafter soluble β) was produced by transfecting NIH 3T3 fibroblasts with the modified β -chain cDNA ("anchor minus" cDNA) lacking the entire DNA sequence encoding both intracytoplasmic and transmembrane domains of the native β -chain.

Construction of the expression vector harboring the anchor minus cDNA which encodes the soluble β - (BCMGNeo-sol. β)

The β -chain cDNA (Fig. 1b) was modified into the anchor minus form for the production of the soluble β . A strategy to generate the expression vector containing the anchor minus cDNA is illustrated in Fig. 9. First, the plasmid pIL-2R β 30 containing the 2.3-kb β -chain cDNA in the CDM8 vector was digested with BssH II and Sma I (all restriction enzymes were purchased from New England BioLabs, Beverly, MA, USA), and a 1.9-kb cDNA fragment (base 58-1967) including the entire coding sequence (base 121-1773) of the β -chain was obtained. After fill-in at the BssH II end, the 1.9-kb cDNA was inserted into a Sma I restriction site of pBluescript SK vector (Stratagene, San Diego, CA, USA). This pBluescript SK- β 1.9 plasmid was then digested with Sty I (restriction sites; base 825, 934 and 1235) and Sma I so that all of the intracytoplasmic and transmembrane regions were deleted, leaving bases 121-840 representing most of the extracellular region intact. Next, a 12-base synthetic linker (New England BioLabs, #1060) containing multiple termination codons (TAG) as well as the recognition sequence for Nhe I was phosphorylated and ligated to the Sty I/Sma I-digested plasmid DNA with T4 DNA ligase. After digestion with the Nhe I to remove excess linker, the DNA was ligated to the SK vector to construct pBluescript SK-sol. β .

This pBluescript SK-sol. β was digested with Sal I and Not I (the restriction sites were located within the polylinker region of the pBluescript SK vector), and the resulting 0.8-kb cDNA fragment encoding the soluble β was isolated. This cDNA fragment was introduced into Xho I/Not I-digested BCMGNeo vector (see Karasuyama et al., J. Exp. Med. 169: 13-25, 1989) containing the cytomegalovirus (CMV) promoter and neomycin-resistance gene to generate the final expression plasmid BCMGNeo-sol. β . The BCMGNeo is a shuttle vector containing 69 % of bovine papilloma virus (BPV) sequences which ensure extrachromosomal replication in mammalian cells. As illustrated in Fig. 10, which illustrates the nucleotide sequence and corresponding amino acid sequence for the native and soluble β , the soluble β cDNA encodes a mature protein consisting of 212 amino acids (aa) accompanied by a signal peptide of 26 aa, while the native β -chain cDNA encodes a membrane protein consisting of a signal peptide (26 aa), extracellular (214 aa), transmembrane (25 aa), and intracytoplasmic (286 aa) domains. The nucleotide and corresponding amino acid sequences for the native and soluble β are also illustrated in Fig. 10.

Transfection of NIH 3T3 fibroblast with BCMGNeo-sol. β and establishment of stable transformants secreting the soluble β

cDNA transfection was performed by the protoplast fusion technique as described in Karasuyama et al. (supra.). Briefly, bacteria, containing the BCMGNeo-sol. β were converted to protoplasts and fused with a murine fibroblast cell line, NIH 3T3 by using polyethylene glycol 2,000 (Wako Chemical Industries, Osaka, Japan). Ten million protoplast-fused NIH 3T3 cells were then seeded in four 24-well plates. Twenty five days after the culture in RPMI 1640 medium containing 10 % fetal calf serum (FCS) and 750 μ g/ml of G418 (Geneticin; Sigma, St. Louis, MO, USA), transformant-cell growth was observed in 60 wells out of 104. When determined by the sandwich enzyme-linked immunosorbent assay (ELISA) as described below, culture supernatants from 18 wells out of 60 were found to be positive for the soluble β . Five clones were established by limiting dilution from a well which gave the highest absorbance in the ELISA, and they were all found to secrete high levels of soluble β (Table I). In contrast, NIH 3T3 cells transfected with the full-length β -chain cDNA (designated 3T3- β 11) did not secrete the β -chain molecule to any extent. In the subsequent studies, we used the clone designated 3T3-B4-14 which secreted the highest amount of the soluble β .

Table 1

Levels of the soluble β in the culture supernatant of NIH 3T3 fibroblasts transfected with BCMGNeo-sol. β	
Culture supernatant	Absorbance at 405 nm in ELISA
3T3-B4-1	1.492
3T3-B4-4	1.301
3T3-B4-7	1.259
3T3-B4-14	1.579
3T3-B4-19	1.533
3T3- β 11	0.052
medium alone	0.072

ELISA for detecting the soluble β

Culture supernatants of transfected cells were screened for the presence of the soluble β by a sandwich enzyme-linked immunosorbent assay (ELISA). In this assay were used two monoclonal antibodies Mik- β 1 and - β 3, supra. and Tsudo et al., Proc. Natl. Acad.Sci. USA, 86: 1982-1986, 1989, which recognize the distinct epitopes on the β -chain; i.e. Mik- β 1 recognises the IL-2 binding site, while Mik- β 3 recognises the epitope not involved in the IL-2 binding. As illustrated in Fig. 11, which is a schematic representation of the Sandwich ELISA Immulon-I microtiter plates (Dynatec, Chantilly, VA, USA) were coated overnight with 50 μ l of Mik- β 3 at 10 μ g/ml in Tris-buffered saline (10 mM Tris-HCl, pH 7.4, 0.15 M NaCl). After discarding excess antibody, unbound sites were blocked by incubating with TBS containing 1 % bovine serum albumin for 1 hour. After washing with TBS containing 0.05 % Tween 20 (T-TBS), 50 μ l of culture supernatants of the transformants were added to the wells and incubated for 1 hour. After washing, 50 μ l of biotinylated Mik- β 1 at 1 μ g/ml were added as the secondary antibody to detect the soluble β bound to the primary antibody, Mik- β 3 on the plate. After 45 minutes incubation and subsequent washing, 50 μ l of alkaline phosphatase-conjugated avidin (Tago, Burlingame, CA, USA) were added. After a 45 minutes incubation, the plates were washed, 100 μ l of p-nitrophenyl phosphate were added, and the absorbance of the wells was determined at 405 nm after 45 minutes.

Apparent molecular weight of secreted soluble β

In order to define the molecular size of the soluble β , the 3T3-B4-14 cells were biosynthetically labeled with 35 S-methionine, and the soluble β immunoprecipitated by the Mik- β 1 mAb from the culture super-

natant. Various amounts of immuno precipitates using the Mik- β 1 and, as control UPC 10 mAb for precipitation were loaded and electrophoresed on an 8 % SDS-polyacrylamide gel. As shown in Fig. 12, when examined by the SDS-polyacrylamide gel electrophoresis (PAGE), the Mik- β 1, but not the control UPC 10 mAb, identified a single species of protein with an apparent Mr of 37,000 in the culture supernatant of 3T3-B4-14 cells. This molecular size is in good agreement with that predicted for the truncated β -chain lacking all of the transmembrane (25 aa) and intracytoplasmic (286 aa) regions.

IL-2 binding ability of the soluble β

It was then investigated whether the secreted form of the β -chain is capable of binding IL-2. To this end, a "competitive" sandwich ELISA was employed. In this assay, the soluble β in the culture supernatant was fixed on the solid phase by the Mik- β 3 mAb, a non-inhibitory mAb for IL-2 binding, so that the putative IL-2 binding site on the β -chain would remain unoccupied. Then, serial dilutions of IL-2 or unlabeled Mik- β 1 were added as competitors for biotinylated Mik- β 1. Fig. 13 shows the results of the competitive sandwich ELISA in which the curve -●-●- is for serial dilutions with unlabeled Mik- β 1 and -o-o- for serial dilutions of IL-2. As shown in Fig. 13 unlabeled Mik- β 1 reduced the absorbance dose-dependently, showing the specificity of this system. Likewise, IL-2 efficiently competed dose-dependently with biotinylated Mik- β 1 for the binding to the soluble β , indicating that the soluble β is indeed capable of binding IL-2.

This competition curve is quite similar to that found for the detergent-solubilised native β -chain from YTS cells which express the β -chain alone, indicating that the affinity of the soluble β to IL-2 is comparable to that of the solubilised native β -chain.

The availability of the genes encoding IL-2R β chains makes it possible to explore novel approaches for the functional studies of the IL-2 system. The receptor structure operating in the IL-2 system is unique in that two structurally distinct membrane molecules, the IL-2R α and IL-2R β chains, both bind IL-2 independently. The series of cDNA expression examples described herein substantiate further the previous notion that the α and β chains constitute the high-affinity IL-2R complex via a non-covalent association of the molecules (18, 37). Thus the peculiarity of this system is the involvement of three intermolecular interactions between one ligand and two distinct receptors. By virtue of the present invention it will now be possible to elucidate functional domains of this unique cytokine receptor system. Mutational analyses of the cloned β chain cDNA may provide clues as to the identification of respective domains involved in ligand binding and association with the α chain. To date, little is known about the cascade of biochemical events triggered by cytokines interacting with their homologous receptors. By the present invention we have demonstrated the presence in the IL-2R β chain of a large cytoplasmic region which most likely is involved in driving the IL-2 signal pathway(s). The particular acidic nuclei found in the cytoplasmic region may suggest coupling to other cytoplasmic signal transducers. Alternatively, in view of a previous report on the presence of IL-2 within the nucleus (33), an intriguing possibility is that the acidic as well as the proline-rich regions of the IL-2R β cytoplasmic component may play a role in activation of the genetic programming. The availability of the expression system in which the cDNA-encoded β chain can deliver growth signals will allow further clarification of the functional domains of the receptor. It is now possible to study the essential role of IL-2 in the development and regulation of the immune system.

The availability of soluble counterparts to the cell surface receptor β -chain should facilitate structural analysis of the β -chain since crystallisation of soluble molecules is more easily accomplished than insoluble ones. The soluble molecules can also be used to neutralise the actual cell surface receptors for studies of the biological functions of the receptors or for therapeutic purposes.

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Claims

1. A recombinant DNA molecule coding for the β -chain of an IL-2 receptor or a portion thereof.
2. A recombinant DNA molecule as defined in claim 1 coding for the β -chain of human or murine IL-2 receptor or a portion thereof.
3. A recombinant DNA molecule characterized by a structural gene having the formula:

ATG

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GCG GCC CCT GCT CTG TCC TGG CGT CTG CCC CTC CTC ATC
 CTC CTC CTG CCC CTG GCT ACC TCT TGG GCA TCT GCA GCG
 GTG AAT GGC ACT TCC CAG TTC AGA TGC TTC TAC AAC TCG
 AGA GCC AAC ATC TCC TGT CTC TGG AGC CAA GAT GGG GCT
 CTG CAG GAC ACT TCC TGC CAA GTC CAT GCC TGG CCG GAC
 AGA CGG CGG TGG AAC CAA ACC TGT GAG CTG CTC CCC GTG
 AGT CAA GCA TCC TGG GCC TGC AAC CTG ATC CTC GGA GCC
 CCA GAT TCT CAG AAA CTG ACC ACA GTT GAC ATC GTC ACC
 CTG AGG GTG CTG TGC CGT GAG GGG GTG CGA TGG AGG GTG
 ATG GCC ATC CAG GAC TTC AAG CCC TTT GAG AAC CTT CGC
 CTG ATG GCC CCC ATC TCC CTC CAA GTT GTC CAC GTG GAG
 ACC CAC AGA TGG AAC ATA AGC TGG GAA ATC TCC CAA GCC
 TCC CAC TAC TTT GAA AGA CAC CTG GAG TTC GAG GCC CGG
 ACG CTG TCC CCA GGC CAC ACC TGG GAG GAG GCC CCC CTG
 CTG ACT CTC AAG CAG AAG CAG GAA TGG ATC TGC CTG GAG
 ACG CTC ACC CCA GAC ACC CAG TAT GAG TTT CAG GTG CGG
 GTC AAG CCT CTG CAA GGC GAG TTC ACG ACC TGG AGC CCC
 TGG AGC CAG CCC CTG GCC TTC AGG ACA AAG CCT GCA GCC
 CTT GGG AAG GAC ACC ATT CCG TGG CTC GGC CAC CTC CTC
 GTG GGC CTC AGC GGG GCT TTT GGC TTC ATC ATC TTA GTG
 TAC TTG CTG ATC AAC TGC AGG AAC ACC GGG CCA TGG CTG
 AAG AAG CTC CTG AAG TGT AAC ACC CCA GAC CCC TCG AAG
 TTC TTT TCC CAG CTG AGC TCA GAG CAT GGA GGA GAC GTC

5 CAG AAG TGG CTC TCT TCG CCC TTC CCC TGA TCG TCC TTC
AGC CCT GGC GGC CTG GCA CCT GAG ATC TCG CCA CTA GAA
GTG CTG GAG AGG GAC AAG GTG ACG CAG CTG CTC CTG CAG
CAG GAC AAG GTG CCT GAG CCC GCA TCC TTA AGC AGC AAC
CAC TCG CTG ACC AGC TGC TTC ACC AAC CAG GGT TAC TTC
10 TTC TTC CAC CTC CCG GAT GCC TTG GAG ATA GAG GCC TGC
CAG GTG TAC TTT ACT TAC GAC CCC TAC TCA GAG GAA GAC
CCT GAT GAG GGT GTG GCC GGG GCA CCC ACA GGG TCT TCC
CCC CAA CCC CTG CAG CCT CTG TCA GGG GAG GAC GAC GCC
15 TAC TGC ACC TTC CCC TCC AGG GAT GAC CTG CTG CTC TTC
TCC CCC AGT CTC CTC GGT GGC CCC AGC CCC CCA AGC ACT
GCC CCT GGG GGC AGT GGG GCC GGT GAA GAG AGG ATG CCC
CCT TCT TTG CAA GAA AGA GTC CCC AGA GAC TGG GAC CCC
20 CAG CCC CTG GGG CCT CCC ACC CCA GGA GTC CCA GAC CTG
GTG GAT TTT CAG CCA CCC CCT GAG CTG GTG CTG CGA GAG
GCT GGG GAG GAG GTC CCT GAC GCT GGC CCC AGG GAG GGA
25 GTC AGT TTC CCC TGG TCC AGG CCT CCT GGG CAG GGG GAG
TTC AGG GCC CTT AAT GCT CGC CTG CCC CTG AAC ACT GAT
GCC TAC TTG TCC CTC CAA GAA CTC CAG GGT CAG GAC CCA
30 ATC CAC TTG GTG TAG

or a portion thereof or a degenerate variant thereof.

4. A recombinant DNA molecule according to claim 1 characterized by a structural gene having the
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ATG

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CCT ACC ATA GCT CTT CCC TGG AGC CTG TCC CTC TAC GTC TTC CTC CTG GCT ACA CCT TGG GCA TCT GCA
 GCA GTG AAA AAC TGT TCC CAT CTT GAA TGC TTC TAC AAC TCA AGA GCC AAT GTC TCT TGC ATG TGG AGC CAT GAA
 GAG GCT CTG AAT GTC ACA ACC TCC CAC GTC CAT GCC AAG TCG AAC CTG CGA CAC TGG AAC AAA ACC TGT GAG CTA
 ACT CTT GTG AGG CAG GCA TCC TGG GCC TGC AAC CTG ATC CTC GGG TCG TTC CCA GAG TCC CAG TCA CTG ACC TCC
 GTG GAC CTC CTT CAC ATA AAT GTG GTG TGC TGC GAA GAG AAG GGT TGG CGT AGG GTA AAG ACC TGC GAC TTC CAT
 CCC TTT GAC AAC CTT CGC CTG GTG GCC CCT CAT TCC CTC CAA GTT CTC CAC AAT GAT ACC CAG AGA TGT AAC ATA
 AGC TGG AAG GTC TCC CAG GTC TCT CAC TAC AAT GAA CCA TAC TTG GAA TTT GAG GCC CGT AGA CGT CTT CTG GGC
 CAC AGC TGG GAG GAT GCA TCC GTA TTA AGC CTC AAG CAG AGA CAG ACG TGG CTC TTC TTG GAG ATG CTG ATC CCT
 AGT ACC TCA TAT CAG GTC CAG GTG AGG GTC AAA GCT CAA CGA AAC AAT ACC GGG ACC TGG AGT CCC TGG AGC CAG
 CCC CTG ACC TTT CGG ACA AGG CCA GAT CCC ATG AAG CAG ATC CTC CCC ATG TCA TGG CTC AGA TAC CTT CTG
 CTG GTC CTT GGT TGT TTT TCT GGC TTC TTC TCC TGC GTC TAC AAT TTG GTC AAG XXX CGC TAC CTT GGG CCA TGG

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CTG AAG ACA GTT CTC AAG TGC CAC ATC CCA GAT CCT TCT GAG TTC TCC CAG CTG AGC TCC CAG CAT GGG GGA
 GAC CTT CAG AAA TGG CTC TCC TCG CCT CTC CCC TTG TCC TTC TCC AGC CCC AGT GGC CCT GCC CAG ATC TCT
 CCG CTG GAA GTG CTC GAC GGA GAT TCC AAG GCC GTG CAG CTG CTC CTG TTA CAG AAG GAC TCT GCC CCT TTA CCC
 TCG CCC AGC GGC CAC TCA CAG GCC AGC TGC TTC ACC AAC CAG GGC TAC TTC TTC CMT CTG CCC AAT GCC TTC
 GAG ATC GAA TCC TGC CAG GTG TAC TTC ACC TAT GAC CCC TGT GTG GAA CAG GAT GAC TAC TGT GCC TTC CCG CCC
 CTG CCC GAG GGA TCT CCC CAC CCA CCT CTG CTG CCT CTG GGA GAA CAG GAT GAC TAC TGT GCC TTC CCG CCC
 AGG GAT GAC CTG CTG CTC TTC TCC CCG AGC CTC ACC CCC AAC ACT GCC TAT GGC GGC AGC AGA GCC CCT GAA
 GAA AGA TCT CCA CTC TCC CTG CAT GAG GGA CTT CCC TCC CTA GCA TCC CGT GAC CTG ATG GGC TTA CAG CGC CCT
 CTG GAG CGG ATG CCG GAA GGT GAT GGA GAG GGG CTG TCT GCC AAT AGC TCT GCG GAG CAG GCC ACT GTC CCA GAA
 GGC AAC CTT CAT GGG CAA GAT CAG GAC AGA GGC CAG GGC CCC ATC CTG ACC CAG GAT GCC TAT CTG TCT
 CTT CAA GAA CTA CAG GCC CAA GAT TCA CTC CAC CTA ATA TAG ***

XXX = GGC or TGC

or a portion thereof or a degenerate variant thereof.

5. A recombinant DNA molecule consisting of the DNA insert of one of the following plasmids

55 pIL-2R β 6,
 pIL-2R β 9,
 pIL-2R β 19,
 pIL-2R β 30,

pMIL-2R β 36,

which codes for IL-2R β or a portion thereof.

6. A recombinant DNA molecule characterised in that it codes for a soluble portion of the entire IL2-R β chain or variant thereof as defined in any one of claims 1 to 5.

7. A recombinant DNA molecule as defined in claim 6, characterised in that it codes for the amino acids about 1 to about 210 of the human IL2-R β chain.

8. A recombinant DNA molecule as defined in claim 7 in which the terminal nucleotides are as follows
GCC CTT GCT AGC TAG

and which codes for a derivative of the water soluble human IL-2R β chain.

9. A recombinant DNA molecule capable of hybridising to the recombinant DNA as defined in any one of the preceding claims and which codes for a protein having the activity of a IL-2R β chain or a soluble portion thereof as defined in claim 6.

10. A recombinant DNA molecule as defined in any one of claims 1 to 9 which further comprises regulatory sequences operably linked to the structural gene for the IL-2 β chain or portion thereof.

11. A recombinant DNA molecule as defined in claim 10 which is a plasmid.

12. The plasmid

pIL-2R β 6,

pIL-2R β 9,

pIL-2R β 19,

pIL-2R β 30 or

pMIL-2R β 36.

13. A host cell which has been transformed by a recombinant DNA molecule as defined in any one of claims 10 to 12.

14. A host cell as defined in claim 13, which is a bacterial cell or a yeast cell or a mammalian cell.

15. A protein having the structure defined by the cDNA according to any one of claims 1 to 9.

16. A hybridoma, sub-clone or mutant thereof capable of secreting a monoclonal antibody having a specific affinity to a protein as defined in claim 15.

17. A monoclonal antibody having a specific affinity to a protein as defined in claim 15.

18. A method of producing a hybridoma as defined in claim 16 which comprises immunizing a non-human animal with a protein as defined in claim 15, removing spleen cells from the immunized animal and fusing the spleen cells with non-immunoglobulin secreting myeloma cells, and selecting from the resulting hybridomas a cell line which produces a monoclonal antibody having the desired binding specificity and, if desired, subsequently sub-cloning said hybridoma.

19. Process for preparing a DNA as claimed in any of the claims 1 to 10 comprising digesting a suitable vector with one or more suitable restriction endonucleases and isolating the desired DNA.

20. A process for preparing a protein as claimed in claim 15 which comprises transforming a suitable host organism with an expression vector containing a coding sequence as claimed in any of the claims 1 to 9 for the desired polypeptide at an appropriate site for expression and isolating the desired protein from the resulting transformants.

21. A process as claimed in claim 20 wherein an expression vector is used as claimed in any of the claims 10 to 12.

22. A process for the preparation of a monoclonal antibody as claimed in claim 17 which comprises cultivating a cell as defined in claim 16 and isolating the monoclonal antibody produced thereby.

23. A process for preparing a host organism as claimed in claim 13 or 14, wherein a vector as claimed in any one of claims 10 to 12 is transformed with a suitable host.

24. A process for preparing a vector as claimed in any one of claims 10 to 12, wherein a DNA-sequence as claimed in any one of claims 1 to 9 is inserted in a suitable vector.

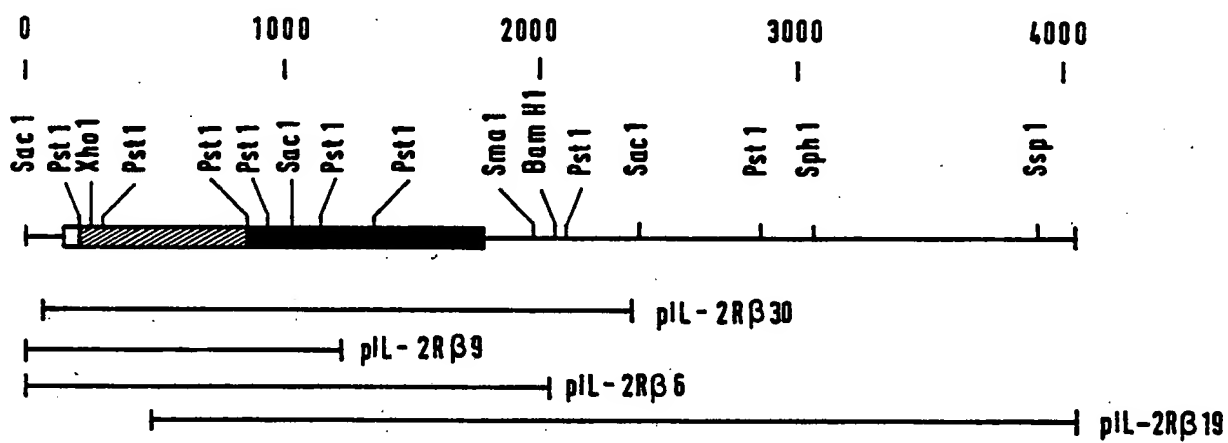


FIG. 1a

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FIG. 1B

PAGE 1

	GCAGCCAGAGCTCAGCAGGGCCCTGGAGAGATGG													34
	CCACGGTCCCAGCACCGGGGAGGACTGGAGAGCGCGCGCTGCCACCGCCCC													
	ATGTCTCAGCCAGGGCTTCCTTCCTCGGCTCCACCCTGTGGATGTA													134
	ATG													Met
	-26													
-25	GCG	GCC	CCT	GCT	CTG	TCC	TGG	CGT	CTG	CCC	CTC	CTC	ATC	173
	Ala	Ala	Pro	Ala	Leu	Ser	Trp	Arg	Leu	Pro	Leu	Leu	Ile	
-12	CTC	CTC	CTG	CCC	CTG	GCT	ACC	TCT	TGG	GCA	TCT	GCA	GCG	212
	Leu	Leu	Leu	Pro	Leu	Ala	Thr	Ser	Trp	Ala	Ser	Ala	Ala	
2	GTG	AAT	GGC	ACT	TCC	CAG	TTC	AGA	TGC	TTC	TAC	AAC	TCG	251
	Val	Asn	Gly	Thr	Ser	Gln	Phe	Thr	Cys	Phe	Tyr	Asn	Ser	
15	AGA	GCC	AAC	ATC	TCC	TGT	CTC	TGG	AGC	CAA	GAT	GGG	GCT	290
	Arg	Ala	Asn	Ile	Ser	Cys	Val	Trp	Ser	Gln	Asp	Gly	Ala	
28	CTG	CAG	GAC	ACT	TCC	TGC	CAA	GTC	CAT	GCC	TGG	CCG	GAC	329
	Leu	Gln	Asp	Thr	Ser	Cys	Gln	Val	His	Ala	Trp	Pro	Asp	
41	AGA	CGG	CGG	TGG	AAC	CAA	ACC	TGT	GAG	CTG	CTC	CCC	GTG	368
	Arg	Arg	Arg	Trp	Asn	Gln	Thr	Cys	Glu	Leu	Leu	Pro	Val	
54	AGT	CAA	GCA	TCC	TGG	GCC	TGC	AAC	CTG	ATC	CTC	GGA	GCC	407
	Ser	Gln	Ala	Ser	Trp	Ala	Cys	Asn	Leu	Ile	Leu	Gly	Ala	
67	CCA	GAT	TCT	CAG	AAA	CTG	ACC	ACA	GTT	GAC	ATC	GTC	ACC	446
	Pro	Asp	Ser	Gln	Lys	Leu	Thr	Thr	Val	Asp	Ile	Val	Thr	
80	CTG	AGG	GTG	CTG	TGC	CGT	GAG	GGG	GTG	CGA	TGG	AGG	GTG	485
	Leu	Arg	Val	Leu	Cys	Arg	Glu	Gly	Val	Arg	Trp	Arg	Val	
93	ATG	GCC	ATC	CAG	GAC	TTC	AAG	CCC	TTT	GAG	AAC	CTT	CGC	524
	Met	Ala	Ile	Gln	Asp	Phe	Lys	Pro	Phe	Glu	Asn	Leu	Arg	
106	CTG	ATG	GCC	CCC	ATC	TCC	CTC	CAA	GTT	GTC	CAC	GTG	GAG	563
	Leu	Met	Ala	Pro	Ile	Ser	Leu	Gln	Val	Val	His	Val	Glu	
119	ACC	CAC	AGA	TGG	AAC	ATA	AGC	TGG	GAA	ATC	TCC	CAA	GCC	602
	Thr	His	Arg	Cys	Asn	Ile	Ser	Trp	Glu	Ile	Ser	Gln	Ala	
132	TCC	CAC	TAC	TTT	GAA	AGA	CAC	CTG	GAG	TTC	GAG	GCC	CGG	641
	Ser	His	Tyr	Phe	Glu	Arg	His	Leu	Glu	Phe	Glu	Ala	Arg	
145	ACG	CTG	TCC	CCA	GGC	CAC	ACC	TGG	GAG	GAG	GCC	CCC	CTG	680
	Thr	Leu	Ser	Pro	Gly	His	Thr	Trp	Glu	Glu	Ala	Pro	Leu	

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FIG. 1B

PAGE 2

158	CTG	ACT	CTC	AAG	CAG	AAG	CAG	GAA	TGG	ATC	TGC	CTG	GAG	719
	Leu	Thr	Leu	Lys	Gln	Lys	Gln	Glu	Trp	Ile	<u>Cys</u>	Leu	Glu	
171	ACG	CTC	ACC	CCA	GAC	ACC	CAG	TAT	GAG	TTT	CAG	GTG	CGG	758
	Thr	Leu	Thr	Pro	Asp	Thr	Gln	Tyr	Glu	Phe	Gln	Val	Arg	
184	GTC	AAG	CCT	CTG	CAA	GGC	GAG	TTC	ACG	ACC	TGG	AGC	CCC	797
	Val	Lys	Pro	Leu	Gln	Gly	Glu	Phe	Thr	Thr	Trp	Ser	Pro	
197	TGG	AGC	CAG	CCC	CTG	GCC	TTC	AGG	ACA	AAG	CCT	GCA	GCC	836
	Trp	Ser	Gln	Pro	Leu	Ala	Phe	Arg	Thr	Lys	Pro	Ala	Ala	
210	CTT	GGG	AAG	GAC	ACC	ATT	CCG	TGG	CTC	GGC	CAC	CTC	CTC	875
	Leu	Gly	Lys	Asp	Thr	<u>Ile</u>	Pro	Trp	Leu	Gly	His	Leu	Leu	
223	GTG	GGC	CTC	AGC	GGG	GCT	TTT	GGC	TTC	ATC	ATC	TTA	GTG	914
	Val	Gly	Leu	Ser	Gly	Ala	Phe	Gly	Phe	Ile	Ile	Leu	Val	
236	TAC	TTG	CTG	ATC	AAC	TGC	AGG	AAC	ACC	GGG	CCA	TGG	CTG	953
	<u>Tyr</u>	Leu	Leu	Ile	Asn	<u>Cys</u>	Arg	Asn	Thr	Gly	Pro	Trp	Leu	
249	AAG	AAG	CTC	CTG	AAG	TGT	AAC	ACC	CCA	GAC	CCC	TCG	AAG	992
	Lys	Lys	Val	Leu	Lys	<u>Cys</u>	Asn	Thr	Pro	Asp	Pro	Ser	Lys	
262	TTC	TTT	TCC	CAG	CTG	AGC	TCA	GAG	CAT	GGA	GGA	GAC	GTC	1031
	Phe	Phe	Ser	Gln	Leu	Ser	Ser	Glu	His	Gly	Gly	Asp	Val	
275	CAG	AAG	TGG	CTC	TCT	TCG	CCC	TTC	CCC	TGA	TCG	TCC	TTC	1070
	Gln	Lys	Trp	Leu	Ser	Ser	Pro	Phe	Pro	Ser	Ser	Ser	Phe	
288	AGC	CCT	GGC	GGC	CTG	GCA	CCT	GAG	ATC	TCG	CCA	CTA	GAA	1109
	Ser	Pro	Gly	Gly	Leu	Ala	Pro	Glu	Ile	Ser	Pro	Leu	Glu	
301	GTG	CTG	GAG	AGG	GAC	AAG	GTG	ACG	CAG	CTG	CTC	CTG	CAG	1148
	Val	Leu	Glu	Arg	Asp	Lys	Val	Thr	Gln	Leu	Leu	Leu	Gln	
314	CAG	GAC	AAG	GTG	CCT	GAG	CCC	GCA	TCC	TTA	AGC	AGC	AAC	1187
	Gln	Asp	Lys	Val	Pro	Glu	Pro	Ala	Ser	Leu	Ser	Ser	<u>Asn</u>	
327	CAC	TCG	CTG	ACC	AGC	TGC	TTC	ACC	AAC	CAG	GGT	TAC	TTC	1226
	<u>His</u>	<u>Ser</u>	Leu	Thr	Ser	<u>Cys</u>	Phe	Thr	Asn	Gln	Gly	Tyr	Phe	
340	TTC	TTC	CAC	CTC	CCG	GAT	GCC	TTG	GAG	ATA	GAG	GCC	TGC	1265
	Phe	Phe	His	Leu	Pro	Asp	Ala	Leu	Glu	Ile	Glu	Ala	<u>Cys</u>	
353	CAG	GTG	TAC	TTT	ACT	TAC	GAC	CCC	TAC	TCA	GAG	GAA	GAC	1304
	Gln	Val	Tyr	Phe	Thr	Tyr	Asp	Pro	Tyr	Ser	Glu	Glu	Asp	
366	CCT	GAT	GAG	GGT	GTG	GCC	GGG	GCA	CCC	ACA	GGG	TCT	TCC	1343
	Pro	Asp	Glu	Gly	Val	Ala	Gly	Ala	Pro	Thr	Gly	Ser	Ser	
379	CCC	CAA	CCC	CTG	CAG	CCT	CTG	TCA	GGG	GAG	GAC	GAC	GCC	1382
	Pro	Gln	Pro	Leu	Gln	Pro	Leu	Ser	Gly	Glu	Asp	Asp	Ala	

FIG. 1B

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PAGE 3

392	TAC Tyr	TGC <u>Cys</u>	ACC Thr	TTC Phe	CCC Pro	TCC Ser	AGG Arg	GAT Asp	GAC Asp	CTG Leu	CTG Leu	CTC Leu	TTC Phe	1421
405	TCC Ser	CCC Pro	AGT Ser	CTC Leu	CTC Leu	GGT Gly	GGC Gly	CCC Pro	AGC Ser	CCC Pro	CCA Pro	AGC Ser	ACT Thr	1460
418	GCC Ala	CCT Pro	GGG Gly	GGC Gly	AGT Ser	GGG Gly	GCC Ala	GGT Gly	GAA Glu	GAG Glu	AGG Arg	ATG Met	CCC Pro	1499
431	CCT Pro	TCT Ser	TTG Leu	CAA Gln	GAA Glu	AGA Arg	GTC Val	CCC Pro	AGA Arg	GAC Asp	TGG Trp	GAC Asp	CCC Pro	1538
444	CAG Gln	CCC Pro	CTG Leu	GGG Gly	CCT Pro	CCC Pro	ACC Thr	CCA Pro	GGA Gly	GTC Val	CCA Pro	GAC Asp	CTG Leu	1577
457	GTG Val	GAT Asp	TTT Phe	CAG Gln	CCA Pro	CCC Pro	CCT Pro	GAG Glu	CTG Leu	GTG Val	CTG Leu	CGA Arg	GAG Glu	1616
470	GCT Ala	GGG Gly	GAG Glu	GAG Glu	GTC Val	CCT Pro	GAC Asp	GCT Ala	GGC Gly	CCC Pro	AGG Arg	GAG Glu	GGA Gly	1655
483	GTC Val	AGT Ser	TTC Phe	CCC Pro	TGG Trp	TCC Ser	AGG Arg	CCT Pro	CCT Pro	GGG Gly	CAG Gln	GGG Gly	GAG Glu	1694
496	TTC Phe	AGG Arg	GCC Ala	CTT Leu	AAT Asn	GCT Ala	CGC Arg	CTG Leu	CCC Pro	CTG Leu	AAC Asn	ACT Thr	GAT Asp	1733
509	GCC Ala	TAC Tyr	TTG Leu	TCC Ser	CTC Leu	CAA Gln	GAA Glu	CTC Leu	CAG Gln	GGT Gly	CAG Gln	GAC Asp	CCA Pro	1772
522	ATC Thr	CAC His	TTG Leu	GTG Val	TAG ***	ACAGATGGCCAGGGTGGGAGGCAGGCAGCT							1817	

GCCTGCTCTGCGCCGAGCCTCAGAAGGACCCTGTTGAGGGTCCTCAGTCCA
 CTGCTGAGGACACTCAGTGTCCAGTTGCAGCTGGACTTCTCCACCCGGATG
 GCCCCACCCAGTCCTGCACACTTGGTCCATCCATTTCCAAACCTCCACTG
 CTGCTCCCGGGTCCTGCTGCCCCGAGCCAGGAAGTGTGTGTGTTGCAGGGGG
 GCAGTAACTCCCCAACTCCCTCGTTAATCACAGGATCCCAACGAATTTAGGC
 TCAGAAGCATCGCTCCTCTCCAGCCCTGCAGCTATTACCAATATCAGTCC
 TCGCGGCTCTCCAGGGCTCCCTGCCCTGACCTCTTCCCTGGGTTTTCTGCC
 CCAGCCTCCTCCTTCCCTCCCCCTCCCGTCCACAGGGCAGCCTGAGCGTGC
 TTTCCAAAACCCAAATATGGCCACGCTCCCCCTCAGTTCAAAACCTTGCAC
 AGGTCCCCTGCCCCTCAGCCCCACTTCTCAGCCTGGTACTTGTACCTCCGG
 TGTCGTGTGGGGACATCCCCTTCTGCAATCCTCCCTACCGTCCTCCCGAGC
 CACTCAGAGCTCCCTCACACCCCTCTGTTGCACATGCTATTCCCTGGGGC
 TGCTGTGCGCTCCCCCTCATCTAGGTGACAACTTCCCTGACTCTTCAAGT

FIG. 1B

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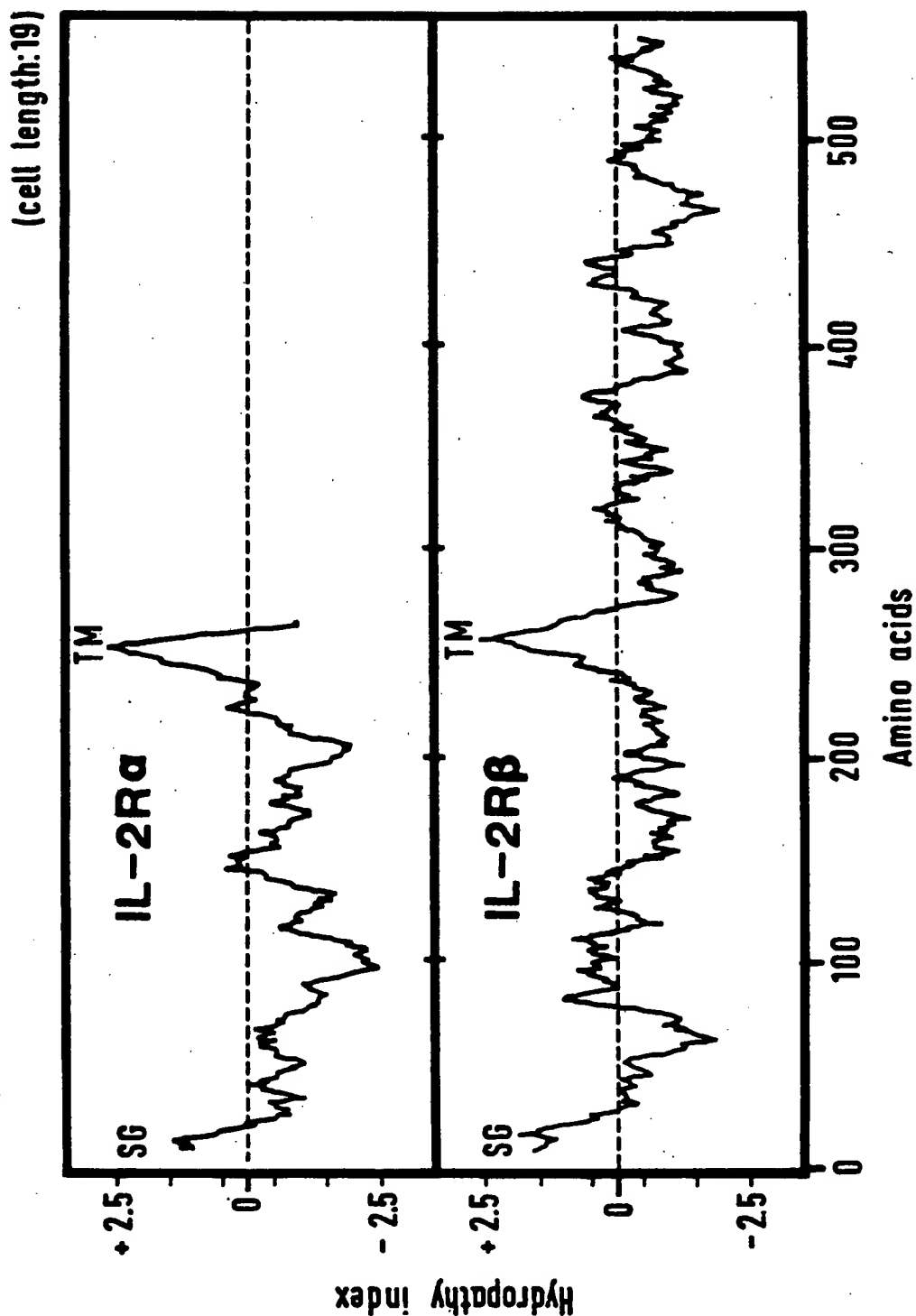
PAGE 4

GCCGGTTTTGCTTCTCCTGGAGGGAAGCACTGCCTCCCTTAATCTGCCAGA
AACTTCTAGCGTCAGTGCTGGAGGGAGAAGCTGTCAGGGACCCAGGGCGCC
TGGAGAAAGAGGCCCTGTTACTATTCTTTGGGATCTCTGAGGCCTCAGAG
TGCTTGGCTGCTGTATCTTTAATGCTGGGGCCCAAGTAAGGGCACAGATCC
CCCCGACAAAGTGGATGCCTGCTGCATCTTCCACAGTGGCTTCACAGACC
CACAAGAGAAGCTGATGGGGAGTAAACCCTGGAGTCCGAGGCCCAGGCAGC
AGCCCCGCTAGTGGTGGGCCCTGATGCTGCCAGGCCTGGGACCTCCCACT
GCCCCCTCCACTGGAGGGGTCTCCTCTGCAGCTCAGGGACTGGCACACTGG
CCTCCAGAAGGGCAGCTCCACAGGGCAGGGCCTCATTATTTTTCACTGCCC
CAGACACAGTGCCCAACACCCCGTCGTATACCCTGGATGAACGAATTAATT
ACCTGGCACCACCTCGTCTGGGCTCCCTGCGCCTGACATTCACACAGAGAG
GCAGAGTCCCGTGCCCATTAGGTCTGGCATGCCCCCTCCTGCAAGGGGCTC
AACCCCTACCCGACCCCTCCACGTATCTTTCCTAGGCAGATCACGTTGC
AATGGCTCAAACAACATTCCACCCAGCAGGACAGTGACCCAGTCCCAGC
TAACTCTGACCTGGGAGCCCTCAGGCACCTGCACTTACAGGCCTTGCTCAC
AGCTGATTGGGCACCTGACCACACGCCCCACAGGCTCTGACCAGCAGCCT
ATGAGGGGGTTTTGGCACCAAGCTCTGTCCAATCAGGTAGGCTGGGCCTGAA
CTAGCCAATCAGATCAACTCTGTCTTGGGCGTTTGAACCTCAGGGAGGGAGG
CCCTTGGGAGCAGGTGCTTGTGGACAAGGCTCCACAAGCGTTGAGCCTTGG
AAAGGTAGACAAGCGTTGAGCCACTAAGCAGAGGACCTTGGGTTCCCAATA
CAAAAATACCTACTGCTGAGAGGGCTGCTGACCATTTGGTCAGGATTCCTG
TTGCCTTTATATCCAAAATAAACTCCCTTTCTTGAGGTTGTCTGAGTCTT
GGGTCTATGCCTTGAAAAAGCTGAATTATTGGACAGTCTCACCTCCTGCC
ATAGGGTCTGAATGTTTCAGACCACAAGGGGCTCCACACCTTTGCTGTGT
GTTCTGGGGCAACCTACTAATCCTCTCTGCAAGTCGGTCTCCTTATCCCCC
CAAATGGAAATTGTATTTGCCTTCTCCACTTTGGGAGGCTCCCACTTCTTG
GGAGGGTTACATTTTTTAAGTCTTAATCATTTGTGACATATGTATCTATAC
ATCCGTATCTTTAATGATCCGTGTGTACCATCTTTGTGATTATTTCTTA
ATATTTTTTCTTTAAGTCAGTTCATTTTCGTTGAAATACATTTATAAAGAA
AAATCTTTGTTACTCTGTAAATGAAAAAACCATTTCGCTATAAATAAAA
GGTAACTGTACAAAATAAGTACAAT

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Nouvellement déposé

FIG. 2



neu eingereicht / Newly filed
Nouvellement déposé

FIG. 3a

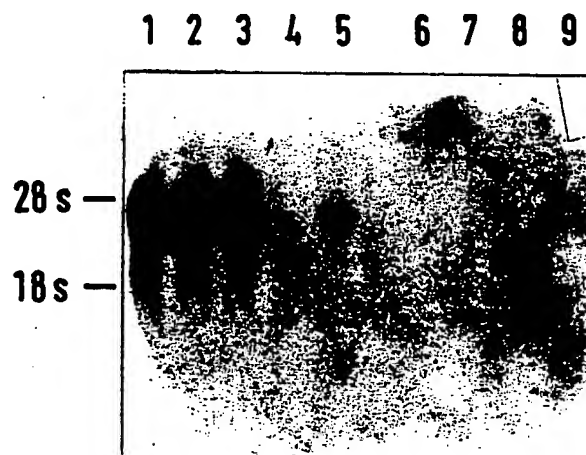
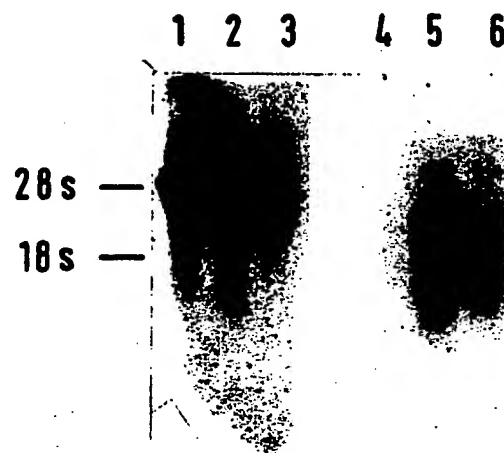


FIG. 3b



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FIG. 4a

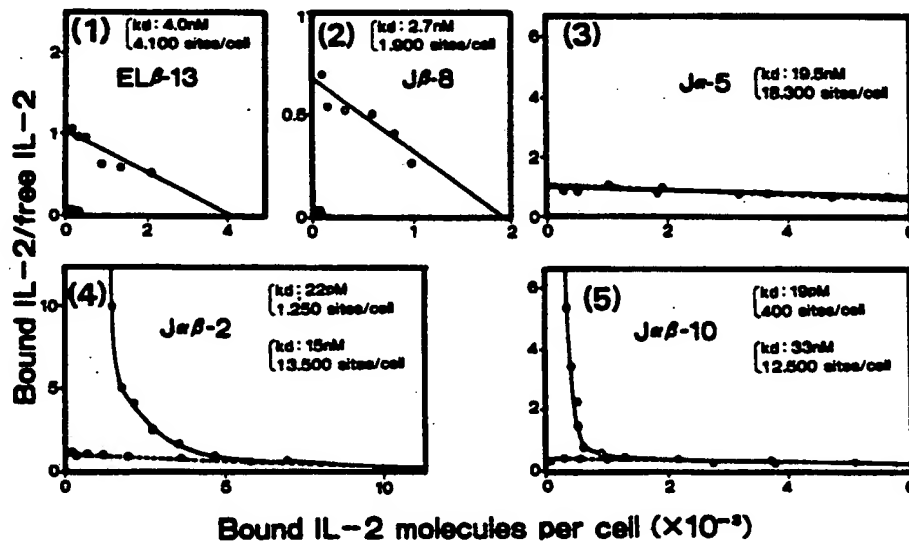
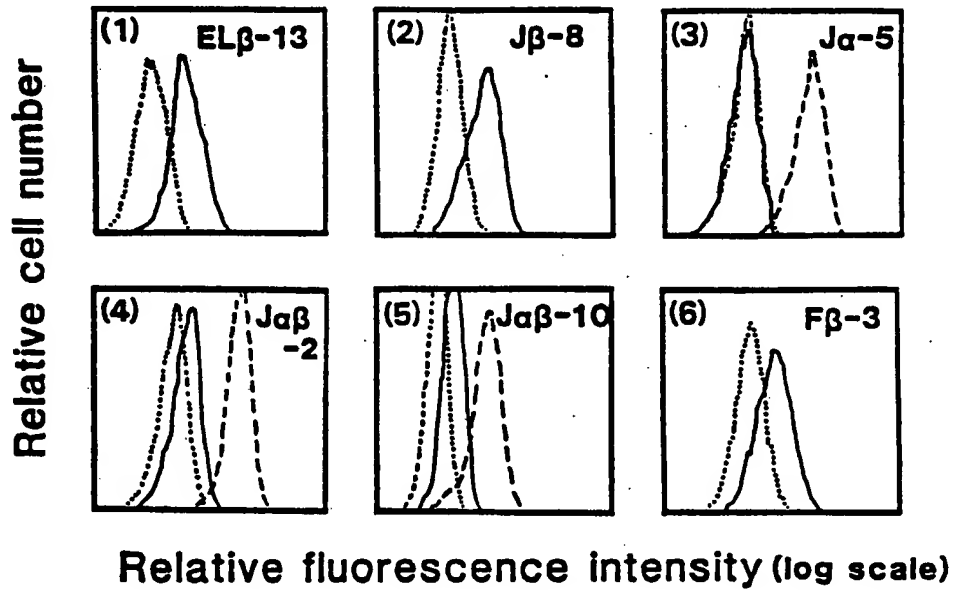
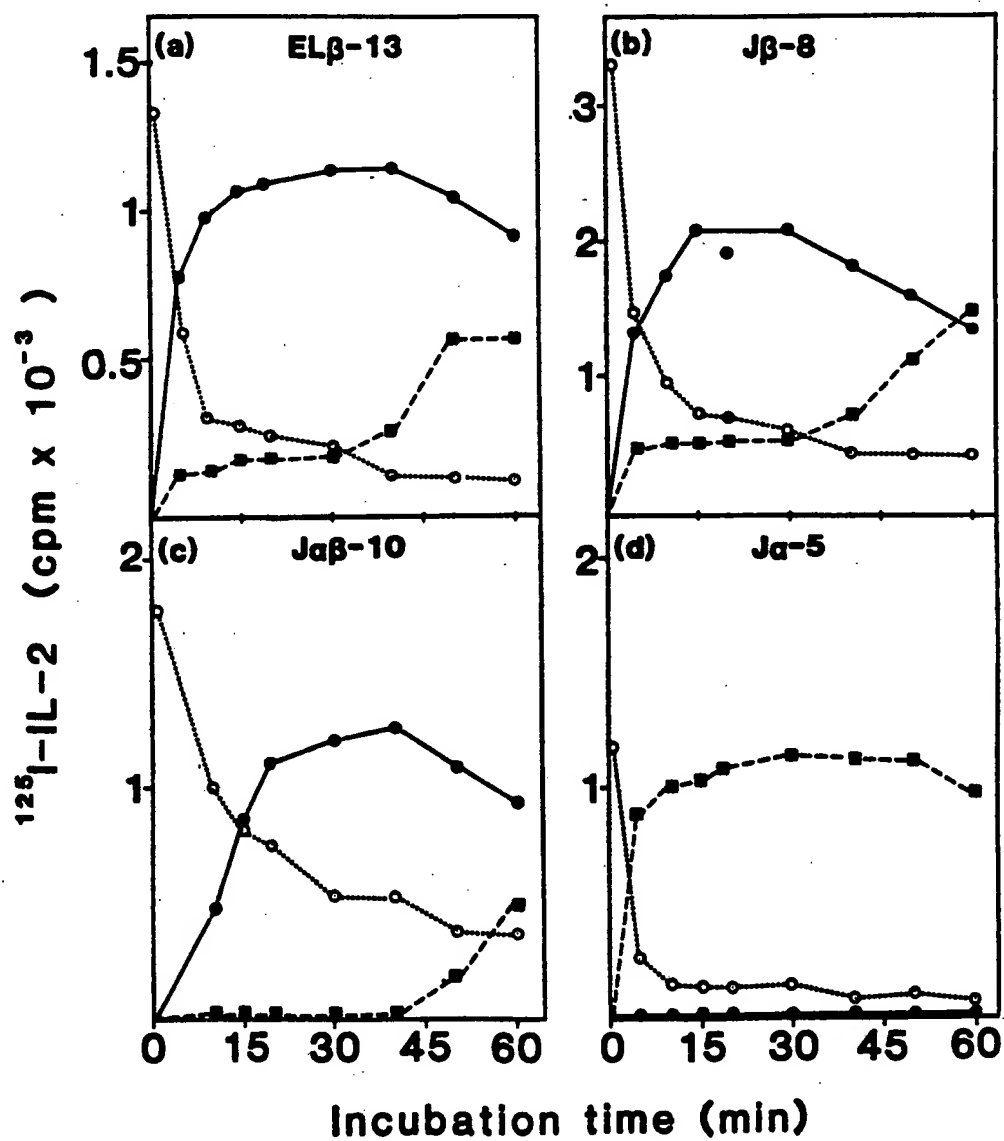


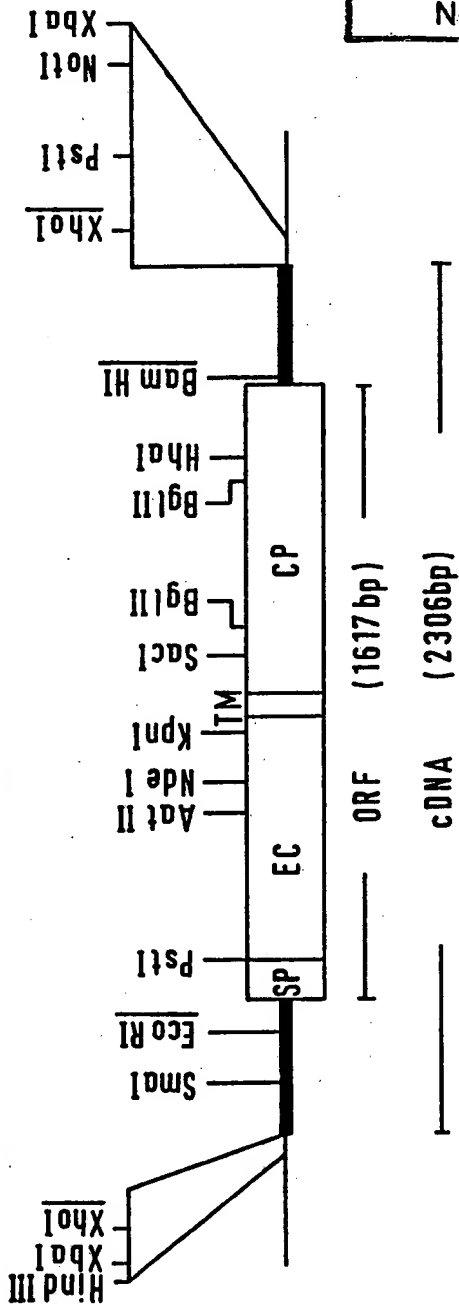
FIG. 4b

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FIG. 6



Restriction enzyme cleavage map of the mouse IL-2
receptor β chain cDNA clone



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Nouvellement déposée

FIG. 7

FIG. 8

251	CTG AAG ACA GTT CTC AAG TGC CAC ATC CCA GAT CCT TCT GAG TTC TCC CAG CTG AGC TCC CAG CAT GGG GGA Leu Lys Thr Val Leu Lys Cys His Ile Pro Asp Pro Ser Glu Phe Phe Ser Glu Ser Ser Gln His Gly Gly	1277
276	GAC CTT CAG AAA TGG CTC TCC TCG CCT GTC CCC TTG TCC TTC TCC AGC CCC AGT GGC CCT GGC ATC TCT Asp Leu Gln Lys Trp Leu Ser Ser Trp Leu Ser Phe Phe Ser Pro Ser Gly Pro Ala Pro Glu Ile Ser	1352
301	CCG CTG GAA GTG CTC GAC GGA GAT TCC AAG GCC GTG CAG CTG CTC CTG TTA CAG AAG GAC TCT GCC CCT TTA CCC Pro Leu Glu Val Leu Asp Gly Asp Ser Lys Ala Val Gln Leu Leu Leu Gln Lys Asp Ser Ala Pro Leu Pro	1427
326	TCG CCC AGC GGC CAC TCA CAG GCC AGC TGC TTC ACC AAC CAG GGC TAC TTC TTC TTC CAT CTG CCC AAT GCC TTG Ser Pro Ser Ser Gly His Ser Gln Ala Ser Cys Phe Thr Asn Gln Gly Tyr Phe Phe Phe His Leu Pro Asn Ala Leu	1502
351	GAG ATC GAA TCC TGC CAG GTG TAC TTC ACC TAT GAC CCC TGT GTG GAA GAG GTG GAG GAG GAT GGG TCA AGG Glu Ile Glu Ser Cys Gln Val Tyr Phe Thr Tyr Asp Pro Cys Val Glu Glu Val Glu Asp Gly Ser Arg	1577
376	CTG CCC GAG GGA TCT CCC CAC CCA CCT CTG CCT CTG GCT GGA GAA CAG GAT GAC TAC TGT GCC TTC CCG CCC Leu Pro Glu Glu Gly Ser Pro His Pro Pro Leu Leu Pro Leu Ala Gly Glu Gln Asp Asp Tyr Cys Ala Phe Pro Pro	1652
401	AGG GAT GAC CTG CTC TCC CCG AGC CTC AGC ACC CCC AAC ACT GCC TAT GGG GGC AGC AGA GCC CCT GAA Arg Asp Asp Leu Leu Leu Phe Ser Pro Ser Leu Ser Thr Pro Asn Thr Ala Tyr Gly Gly Ser Arg Ala Pro Glu	1727
426	GAA AGA TCT CCA CTC TCC CTG CAT GAG GGA CTT CCC TCC CTA GCA TCC CGT GAC CTG ATG GGC TTA CAG CGC CCT Glu Arg Ser Pro Leu Ser Leu His Glu Gly Leu Pro Ser Leu Ala Ser Arg Asp Leu Met Gly Leu Gln Arg Pro	1802
451	CTG GAG CGG ATG CCG GAA GGT GAT GGA GAG GGG CTG TCT GCC AAT AGC TCT GGG GAG CAG GCC AGT GTC CCA GAA Leu Glu Arg Met Pro Glu Gly Asp Gly Glu Gly Leu Ser Ala Asn Ser Ser Gly Glu Gln Ala Ser Val Pro Glu	1877
476	GGC AAC CTT CAT GGG CAA GAT CAG GAC AGA GGC CAG GGC CCC ATC CTG ACC CTG AAC ACC GAT GCC TAT CTG TCT Gly Asn Leu His Gly Gln Asp Gln Asp Arg Gly Gln Gly Pro Ile Leu Thr Leu Asn Thr Asp Ala Tyr Leu Ser	1952
501	CTT CAA GAA CTA CAG GCC CAA GAT TCA GTC CAC CTA ATA TAG CAGGTGGCCAGGACTGGGATCCAGCTGCCTGGATCAGGTCAG Leu Gln Glu Leu Gln Ala Gln Asp Ser Val His Leu Ile ***	2036
GCTTGAGGAAGACTGCTTAAGAGGTCTCTTGAGGACAGTCCACTGCTGGCTATCCCTGCCCCCCCCCTCCAAACITTAATCATCCACT TCTGAACCTCCATTGCTACTTCTCTGGTCTAACCCAGGTTTGGTGAGGGTGGGAGTGGGGAGCGGTGGTCAAGCTCCACTGCCCTATTAGTCATGAG GTCAC TAGCTTCTGTACCTGCCTCCTCCCTCTGCTTGCCTTCACAGGGCAGCTAGAACTTGCTTCCCCC		2135 2234 2306

XXX = GGC or TGC

Y = Gly or Cys

FIG. 8

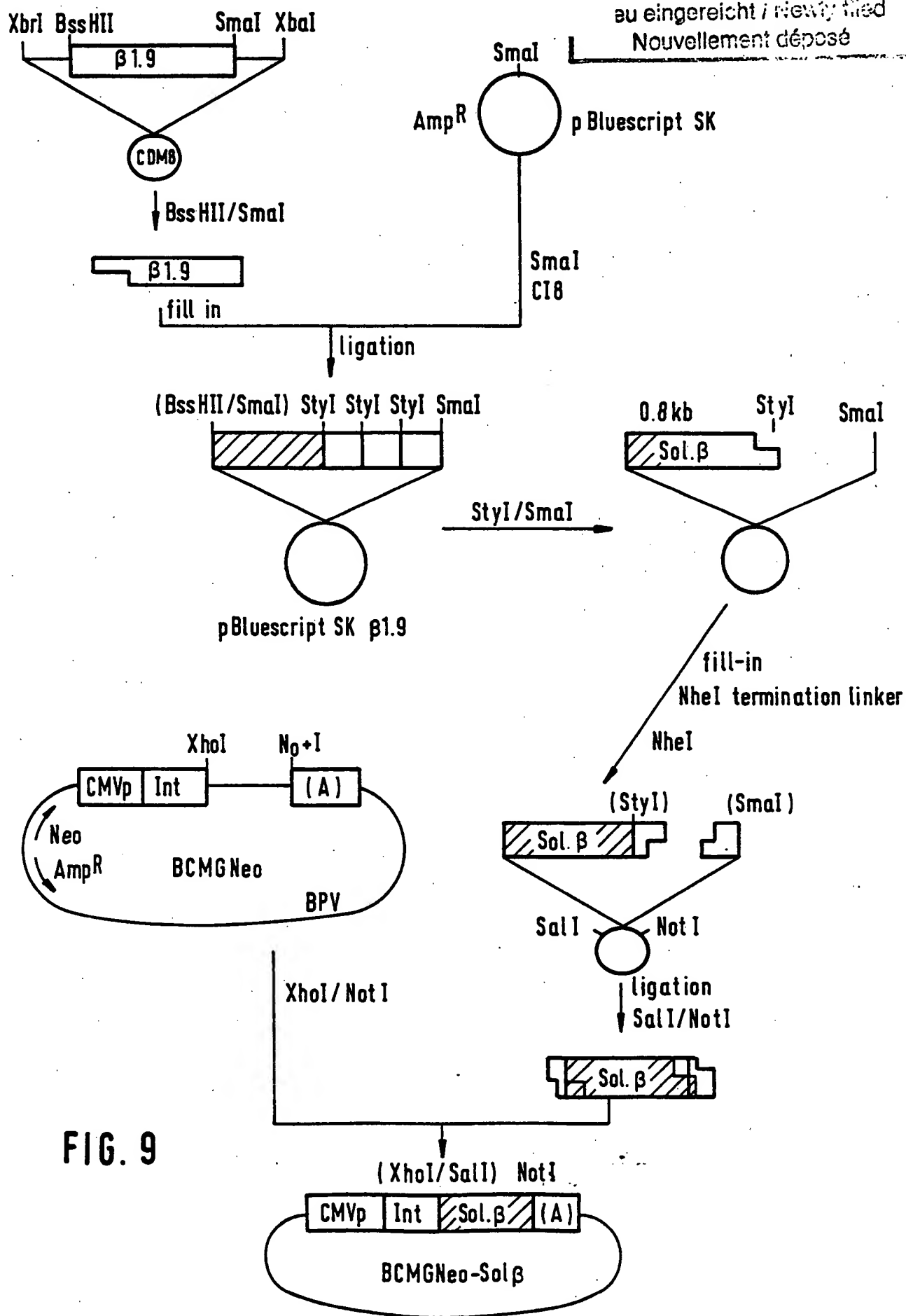
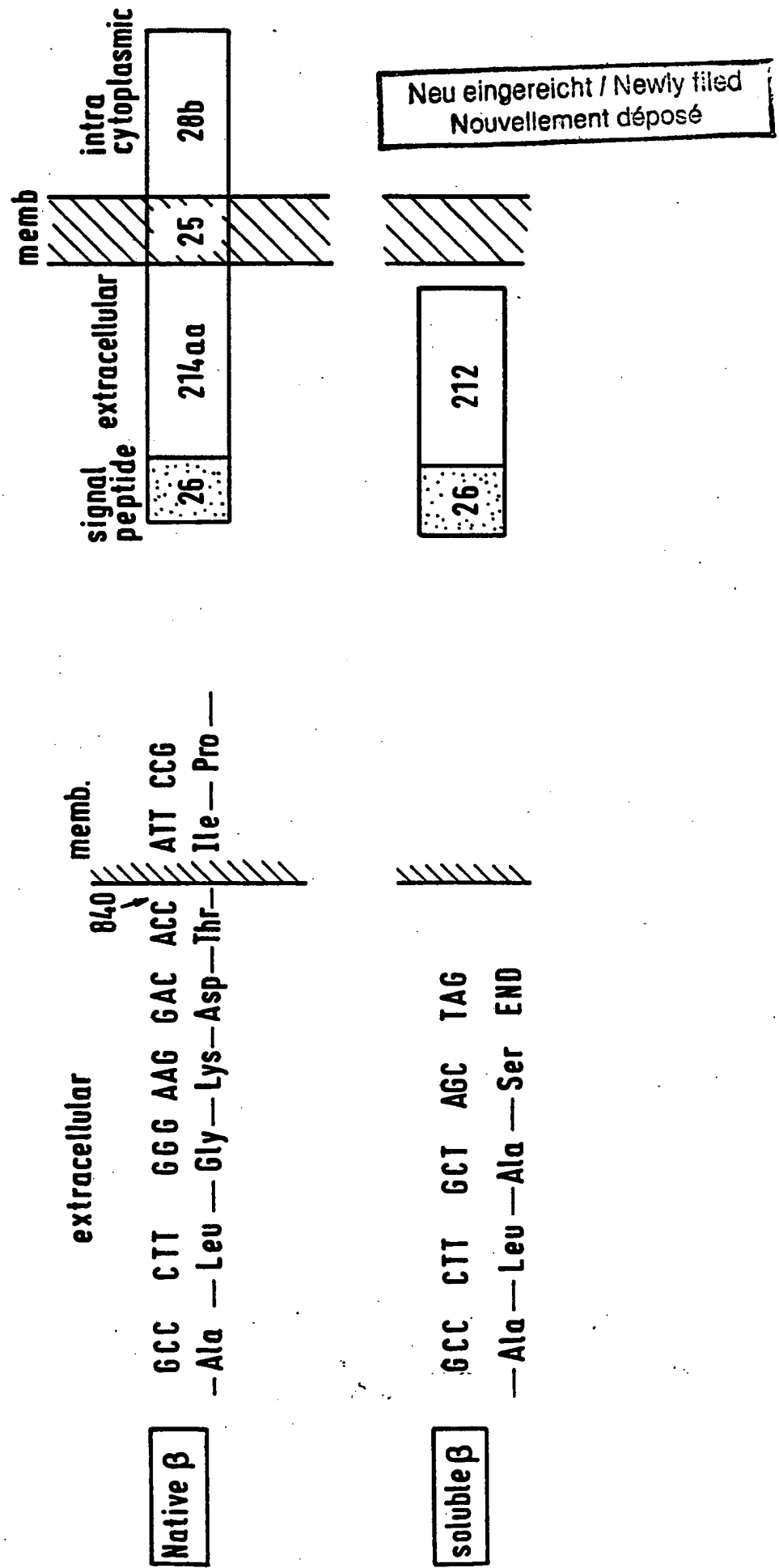


FIG. 9

FIG. 10



neu eingereicht / Newly filed
Nouvellement déposé

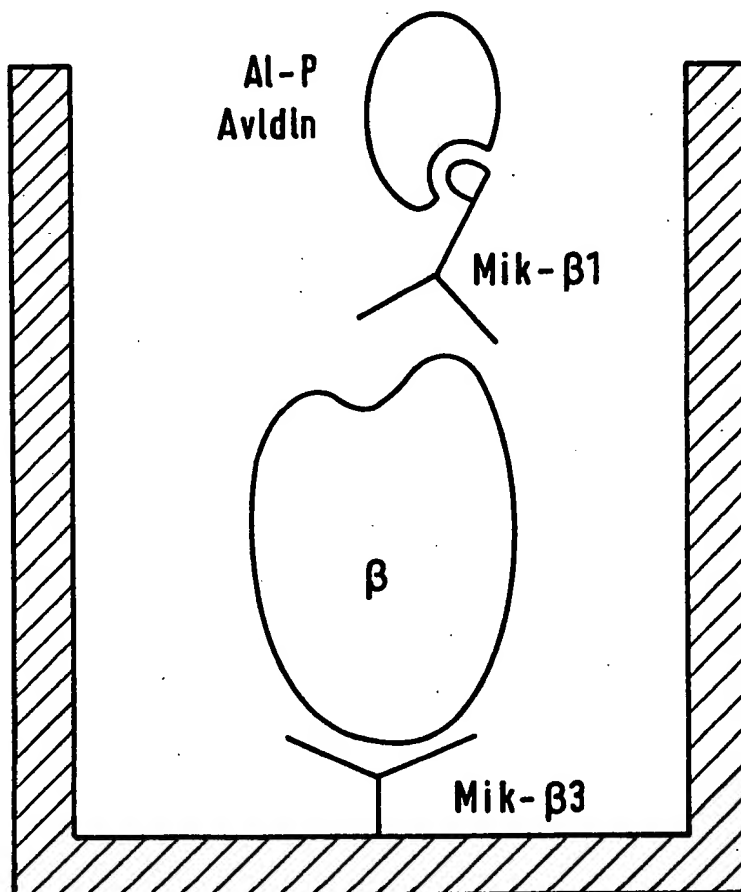


FIG. 11

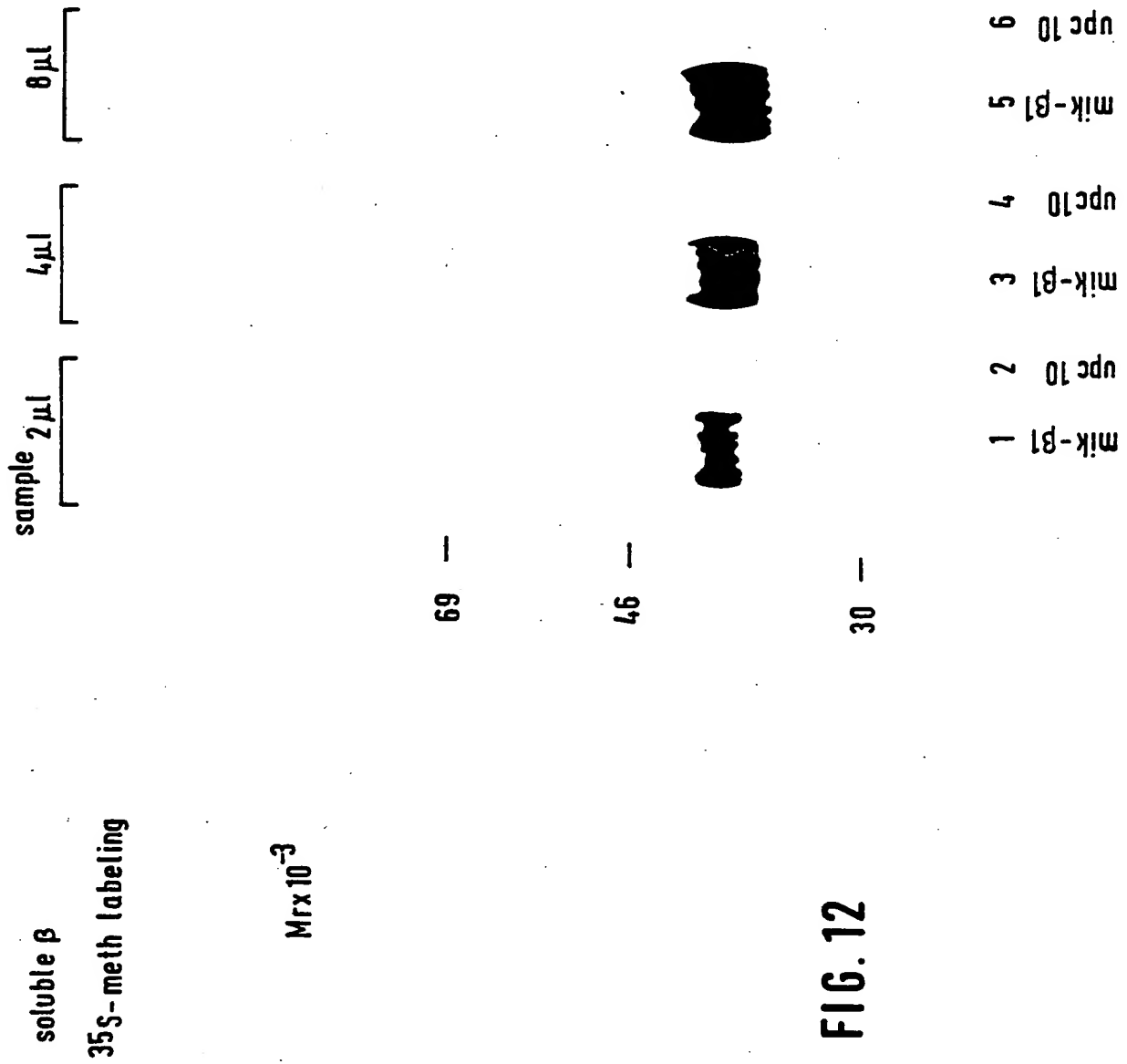
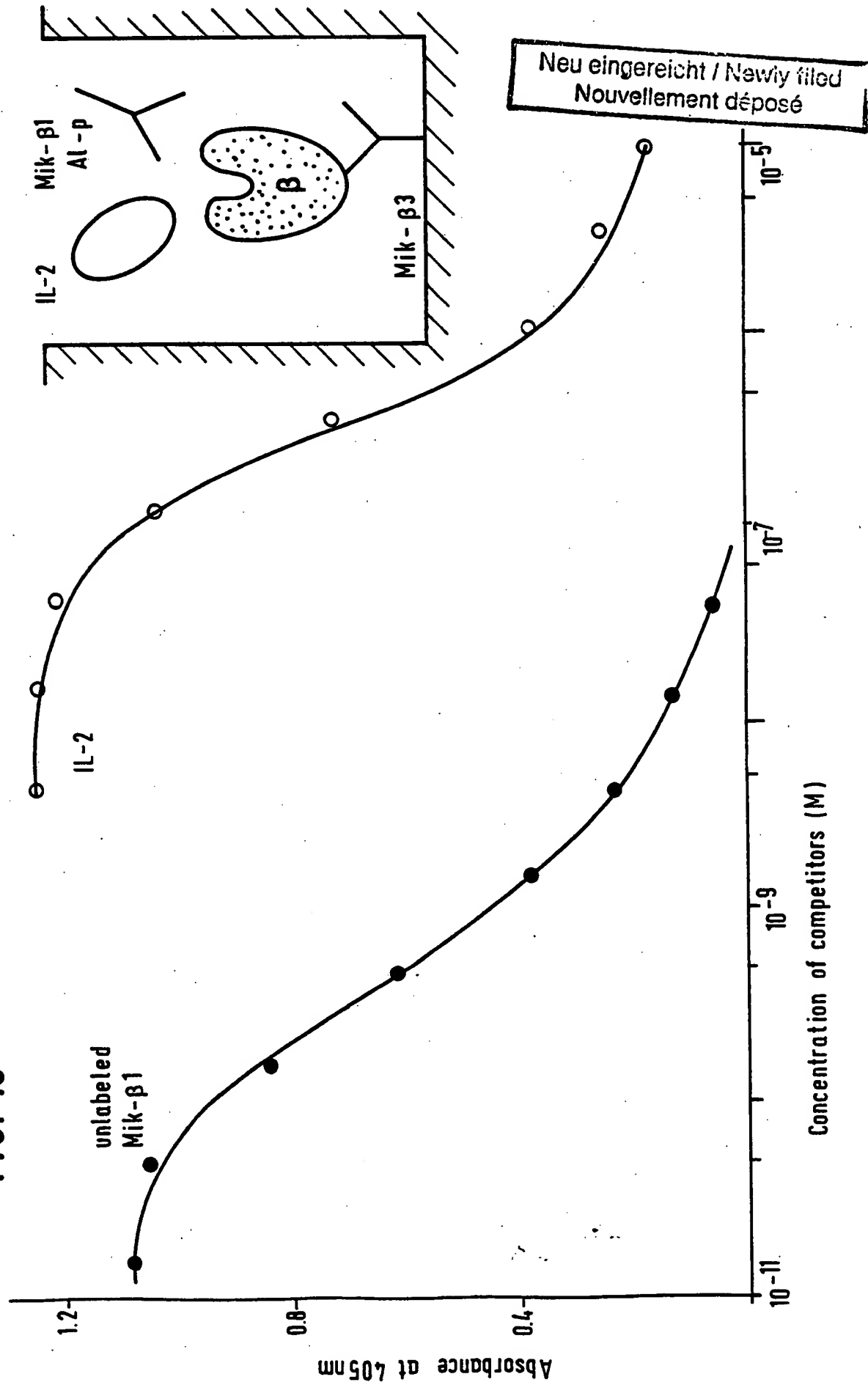


FIG. 12

neu eingereicht / Newly filed
 Nouvellement déposé

FIG. 13





DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
X	WO-A-8 900 168 (THE UNITED STATES OF AMERICA) * Page 6, line 1 - page 8, line 30 *	15-18, 22	C 12 N 15/12 C 12 P 21/08 C 12 P 21/02
Y	---	1-14, 19-21, 23, 24	
Y	EP-A-0 162 699 (IMMUNEX CORP.) ---	1-14, 19-21, 23, 24	
P, X	SCIENCE, vol. 244, 5th May 1989, pages 551-556, Washington, DC, US; M. HATAKEYAMA et al.: "Interleukin-2 receptor beta chain gene: Generation of three receptor forms by cloned human alpha and beta chain cDNA's" * Whole document *	1-24	
P, X	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCE, USA, vol. 86, March 1989, pages 1982-1986, Washington, DC, US; M. TSUDO et al.: "Characterization of the interleukin 2 receptor beta chain using three distinct monoclonal antibodies" * Page 1982: "Materials and methods" *	22	TECHNICAL FIELDS SEARCHED (Int. Cl.5) C 12 N C 12 P
P, X	THE JOURNAL OF IMMUNOLOGY, vol. 143, no. 12, 15th December 1989, pages 4039-4043, The American Association of Immunologists, US; M. TSUDO et al.: "Reconstitution of a functional IL-2 receptor by the beta-chain cDNA, a newly acquired receptor transduced negative signal" --- -/-	6	
The present search report has been drawn up for all claims			
Place of search THE HAGUE		Date of completion of the search 17-06-1990	Examiner VAN PUTTEN A.J.
CATEGORY OF CITED DOCUMENTS X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document			



DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
A	THE JOURNAL OF IMMUNOLOGY, vol. 136, no. 11, 1st June 1986, pages 4099-4105, The American Association of Immunologists, US; B.F. TREIGER et al.: "A secreted form of the human interleukin 2 receptor encoded by an "Anchor minus" cDNA" ---	1-24	
X	IMMUNOBIOLOGY, vol. 175, no. 4, 1987, pages 343-344, Stuttgart, DE; G. KRUPPA et al.: "Transfer and expression of the human IL 2 receptor beta-chain gene in murine fibroblasts and T lymphoid cells using retroviral vectors" * Abstract * ---	1	
A	NATURE, vol. 327, no. 6122, 11th-17th June 1987, pages 518-522, Basingstoke, Hampshire, GB; M. DUKOVICH et al.: "A second human interleukin-2 binding protein that may be a component of high-affinity interleukin-2 receptors" -----		TECHNICAL FIELDS SEARCHED (Int. Cl.5)
The present search report has been drawn up for all claims			
Place of search THE HAGUE		Date of completion of the search 17-06-1990	Examiner VAN PUTTEN A.J.
CATEGORY OF CITED DOCUMENTS X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons ----- & : member of the same patent family, corresponding document			